

Crisis leads to change

THE outlook from every capital city must at present be bleak and yet nowhere can the immediate prospects be more depressing than in London. Although the rise in the price of oil and restrictions on its supply have hit all countries and the long-term problems of price are terribly serious for developing countries, the industrialised nations are the first to feel the pinch. Britain with its natural gas and coal resources should be marginally better equipped than most other European countries to cope with oil shortages, but it has contrived to compound shortages by virtue of two industrial actions—of the coal miners and of railway engine drivers.

The miners present the bigger problem. Their action has the deeper impact on the country over the longer term and the first effects are now being seen. There is little doubt, despite the inevitable and rather silly political sparring, that coal supplies were falling to a dangerous level and electricity supplies would have been jeopardised by early February. To avoid this the government has imposed a three-day working week throughout industry. The industrial chaos that this is rapidly leading to is deemed necessary in order to maintain vital services up to the end of the winter. It is obvious that one of the purposes of the three-day week has been to prevent coal supplies being run down, as they were in 1972, to a level at which the miners could strike and get exactly what they wanted. There is considerable public sympathy for the miners, whose pay structure contains glaring anomalies.

The government meanwhile pursues its implacable course. Stage Three of its anti-inflation policy, originally heralded as offering flexibility in the negotiation process, is only three months old and seems to offer no negotiating possibilities. Unions have determined to ignore Stage Three in putting in wage claims and the situation is dead-lock—so much so that to the exasperation of the public, meetings between the sides are rare, as there is nothing to talk about. During this sleep of reason, the participants seem to have allowed themselves to be saddled with two burdens which sensible negotiators could have avoided—lack of fall-back positions and problems of serious loss of face in the event of failure.

As a result of this, whereas all countries are experiencing hardships, Britain is in danger of falling apart.

The political scene will perhaps emerge as the most damaged. For the past two or three years politicians the world over have been dismayed by the rise in world commodity prices which has taken from them much of their ability to control national economies. This in itself need not discredit the politicians provided a certain amount of frankness is maintained about what governments can and cannot do. Frankness is not, however, a mark of the British politician. Political posturing over inflation has now also become posturing over energy and the three-day week. There is a growing disbelief in the country that either major political party can be turned

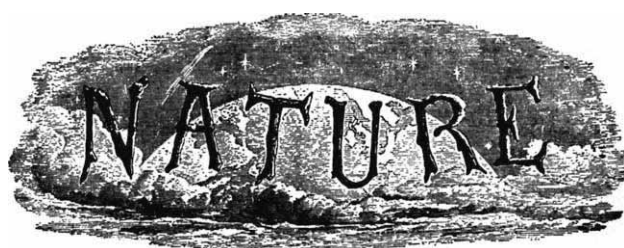
to in a crisis like this for a cool and lasting solution.

Socially, the danger signals are beginning to appear. The longer-term consequences of the present crisis—a depressed standard of living, less expenditure on desirable projects and so on—are not yet at hand but the short-term indicators, such as the way people behave, are ominous. Just as the bad economic times of the 1920s and 1930s left a mark on the personality of a generation, so the present trauma of crisis, confrontation, divisiveness and inflation may leave a scar of bad temper and distrust.

The military impact of the energy crisis for countries such as Britain lacking local oil supplies is worth comment. It does not seem likely that any such nation will be able to conduct warfare which is displeasing to oil producers without being quickly throttled. The contrast with Suez in 1956 when the retaliation was short-lived is striking. If the crisis thus simply imposed more restraints on military ventures it could be reckoned a good thing. Power to prevent, however, also implies power to promote. Only time will tell whether oil producers can dictate the military activities of other nations.

What are the prospects for science? As we report elsewhere the first impact of budget cuts is now being felt in research spending. This budget was produced before it was clear that the three-day week was a relatively long-term affair. Another month of this and further cuts in public spending may be necessitated. Customer-oriented science is probably a less attractive target for cuts than pure science. Mr Barber's razor may do some of Lord Rothschild's work.

100 years ago



THE POLLUTION OF RIVERS

The question which has to be settled is not whether anything is to be done to remedy the certainly disgraceful state of some of our streams, but rather to what extent can the purification be pushed without detriment to the industry of the district; and when this has been decided comes the next question, how this partial purification is to be effected. That it can only be a partial purification is clear from the conclusions of the Commissioners themselves, who do not propose any plan by which the water of our rivers, in populous districts at present little better than sewers, shall be so purified as to be fit for drinking purposes.

From *Nature*, 9, 197, January 15, 1874.



international news

Energy planning Soviet style

by our Soviet correspondent

ENERGY has been an emotive concept in the Soviet Union since 1920, when the Eighth All-Russian Congress of Soviets adopted the "Goelro" plan for the electrification of the country, with Lenin's famous equation "Communism = Soviet Power + Electrification". It was a coal miner called Stakhanov who, in 1935, by mining fourteen times the normal output of one man in a single shift, became the eponymous hero of the concept of 'Stakhanovite' labour. New prestige projects, such as the Bratsk power station or the Novopolotsk oil refinery, remain standard subjects for poets of a high literary reputation—and their works are analysed by the critics with more than usual attention to their political content. Yet the very significance of energy as a touch-stone of Soviet progress makes it somewhat difficult to assess the overall situation. When the opening of each new power station is reported by the press in careful detail, and with the Soviet propensity for presenting statistics not in absolute figures, but as percentages relative to some inaccessible base year, it is easy to become lost in a mass of minutiae on the one hand and of vague generalities on the other.

Conversely, it is not always easy to distinguish the economically viable project from that undertaken primarily for prestige purposes. Thus the plan described in an *Izvestiya* interview in January 1971 by Minister of Electrification Petr Neporozhnyi, which involved the use of power stations on the White Sea to fill in at peak consumption periods, loses a certain amount of its credibility when one remembers that they would be inoperable during the winter months—at precisely the time when the new unified grid has to depend almost entirely on thermal generation since the Siberian hydroelectric stations are also icebound. On the other hand, the imaginative proposals of I. V. Shishkin for running an entire city on thermal waters would seem to be economically viable, given his basic assumptions of a stratum temperature of 150° C and a sufficient water table (*Priroda*, No. 5, 95–103; 1969).

The present power difficulties of the western nations, however, have evoked considerable comment in the Soviet press from which the picture does become a little clearer. Side by side with cartoons of the pathetic British motorist walking his car to work emerge more factual statements, such as an interview (*Novoe Vremya*, No. 48, 20–22; 1973) with Ruben Napoleonovich Andreasyan, a senior researcher at the Institute of World Economics and International Relations. Andreasyan categorically denied Western reports of an oil shortage in the Soviet Union and was prepared to quote figures. The Soviet Union, he stated, "is one of the richest countries in the world as regards resources of liquid fuel. . . It should be said that the oil of Western Siberia is outstanding not only on account of its high quality, but also its low cost. . . The Soviet Union is already one of the largest oil exporters in the world. In 1972, it exported 76.2 million tonnes of crude oil, not counting prepared petroleum products". As to the reports that the Soviet Union imports Arab oil, this is true, but the amount involved is only 7.8 million tonnes, ten times less than the export figure, and is intended as a gesture of aid to the balance of payments of the oil exporting countries. Of the oil exported, some 50 million tonnes goes to the non-Communist world, and the rest to the Comecon bloc through the "Druzhiba" pipeline system, which celebrated its tenth anniversary on December 23, 1973. Reports of the economic integration of Comecon would suggest that by now the smaller member countries supplement their domestic oil production (if any) entirely from Soviet sources, but Reuter reports from Warsaw early in the current crisis (November 25) revealed that Poland, at least, still buys a significant amount of her oil on the world market and has now introduced a speed limit of 80 kilometres an hour and similar measures for saving fuel.

Soviet oil production figures for January to November 1973 (*Pravda*, December 11, 1973) totalled some 385,000 million tonnes (25 million tonnes more than for the same period in 1972), together with some 46,000 cubic metres of natural and petroleum gas. The pride of the Soviet oil industry, the West Siberian (Tyumen') oil field (opened up in 1964), has already surpassed its target for the current year by some 2.3 million

tonnes and is expected to produce 87.5 million tonnes in all—some 3 million tonnes above the target. According to a Tass report headlined by the Minsk newspaper *Zviazda*, the Tyumen' field has achieved a record output of 285,000 tonnes in 24 hours.

Nevertheless, the budget and state plan for 1974 (*Pravda*, December 13, 1973) places great stress on "the fuel and energy sectors". The output of electrical power is assigned a target of 975,000 million kilowatt hours (kWh)—an increase of 6.6% in comparison with 1973. The output from nuclear power stations is to be increased to 161,000 million kWh (38% more than in 1973). This increase in electrical output will permit industry to increase its consumption by 5.7%, agriculture by 13% and "the community and living needs of the urban population" by 8%.

The following increases in fossil fuel outputs are envisaged: petroleum and gas condensates by 30 million tonnes (7%), gas by 20.8 million cubic metres (1.8%), mainly from West Siberia, the Kazakh SSR, Central Asia, the Komi ASSR, the Udmurt ASSR and the Perm' and Orenburg regions. To serve these fields, more than 9,000 km of new pipeline and 85 pumping and compressor stations are scheduled to go into service during 1974. The oil refining and petrochemical industries are assigned an increased target of 6.8% with a "considerable increase" in the output of high-octane and low-sulphur fuels. A mouthwatering picture to the harassed Western consumer, perhaps—yet it must be recalled that one has no clear picture of the potential demand, and plans for such large increases may be evidence of the need to close even greater gaps.

Coal output is planned to be 679.1 million tonnes—10 million tonnes more than 1973, with 8 million tonnes of the increase coming from open-cast mining. This emphasis on open-cast mining became evident in the late 1950s with the opening up of new coalfields in northern Kazakhstan and central Siberia.

These figures, it is stated, should basically cover all "normal regular demands". Nevertheless, "it is necessary to exhibit care in expending fuel resources, and to ensure the unconditional fulfilment of the plans for saving fuel and electricity"; this is another indication, perhaps, that demand still outstrips supply.

Geothermal energy in California

Colin Norman, Washington

LATER this month the US Department of the Interior will take a large step towards opening up a new source of energy in the United States. On January 22 some 50,000 acres of federal land in California will be leased to private industry so that a start can be made on producing electricity from steam and superheated water trapped in deposits relatively close to the surface. Such deposits of geothermal energy have long been touted as potential sources of power, and the energy crisis has provided the necessary incentive for the federal government to set aside environmental and other concerns and open up large areas of the western United States for drilling to tap the resources.

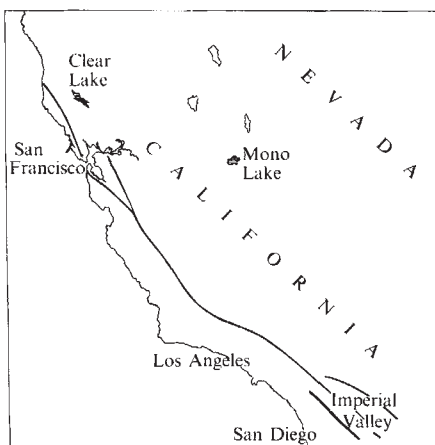
The potential impact of geothermal energy on power supplies in the United States is a matter of some debate, for estimates range from 1% of energy supplies by the year 2000 to a staggering 20%. A report put together by the University of Alaska under the direction of Walter J. Hickel, former Secretary of the Interior, came up with the prediction earlier this year, for example, that geothermal energy could provide 1,000 million megawatt hours (MWh) of electricity by 1985, and 3,100 million MWh by the end of the century—more than five times the present power demand of the whole of New England. The Department of the Interior, on the other hand, reckons that by the year 2000, geothermal energy may be supplying a modest (but important) 2% of the United States national electricity generating capacity.

In any case, commercially exploitable geothermal resources are regions where the great heat of the Earth's interior is close to the surface. Essentially, that means along the edges of plates and near recent volcanic formations. Since two plates meet at the San Andreas fault in California, and since much of the Western United States consists of volcanic rocks, the potential geothermal resources could be large.

The idea behind production of electricity from geothermal resources is simple enough—in fact, a geothermal power plant has been operating at Larderello in Italy since 1904 and another plant has recently been built in northern California. The easiest deposits to exploit are entrapments of hot, dry steam, which can be tapped simply by sinking a pipe into the deposit and using the steam directly to drive turbines. Deposits of superheated water at about 200° C are also relatively easy to tap, since the water can be flashed to steam by relieving some of the pressure.

Unfortunately, however, such deposits

are relatively few and far between, and geothermal energy will have a large impact on power supplies only if methods can be found for exploiting cooler water deposits and hot rock reservoirs. As far as the former is concerned, it may be possible to transfer heat from the water to a more volatile liquid and use the vapour from the second liquid to drive a turbine in a closed system. Hot rock reservoirs are by far the most abundant geothermal resources but they are also the most difficult to exploit. Research is under way on methods which involve fracturing the rock underground with explosives and circulating a liquid through the fractured rock to pick up some of the heat. But commercial application of such techniques is considered to be a long way off.



Map showing locations of areas to be leased.

The areas which the Department of the Interior will lease this month all contain steam and superheated water deposits and thus can be used to generate electricity fairly quickly. Three areas will be leased. One, the Clear Lake-Geysers area in northern California, is already producing some 400 MW of electricity and is reckoned to be capable of producing between 1,000 and 5,000 MW. The second area is in the Mono Lake-Long Valley region east of San Francisco; this is expected to produce about 1,750 MW. And the third area is the Imperial Valley, in southern California, along the Mexican border. This field has the largest potential, for it has been estimated that it could produce between 3,000 and 30,000 MW of electricity, and it also lies close to Los Angeles and other large urban centres.

In addition to opening up those areas for geothermal power production, the Department of the Interior has announced that it will accept bids from private industry for other federal lands which may be potential geothermal resource areas. This should stimulate the search for other areas of geothermal

energy which can be easily exploited. And, for the longer term, the federal government is preparing to step up spending on research and development on production of power from less accessible geothermal resources. The five-year plan put together by Dr Dixie Lee Ray, Chairman of the Atomic Energy Commission, envisages an expenditure of some \$185 million before 1980, for example.

Agreement on budget for HEW

Colin Norman, Washington

CONGRESS and the Administration have at last managed to reach agreement on the size of the budget for the Department of Health, Education and Welfare (HEW). The agreement will give the research institutes of the National Institutes of Health at least \$1,723 million this fiscal year—nearly \$200 million more than the Administration had wanted to spend. A number of recent court decisions have also sharply reduced the Administration's ability to hold back money appropriated by Congress and thus it seems unlikely that the Administration will be able to follow its usual tactics of simply refusing to spend extra money voted by Congress.

The compromise budget was passed by Congress early last month and it was signed into law by President Nixon just before Christmas. The fact that a budget has at least been agreed upon is a landmark, for HEW has been operating for more than 18 months on a complicated budgetary formula because Congress and the Administration have repeatedly failed to see eye to eye on spending priorities. Last year, President Nixon vetoed two HEW appropriations bills passed by Congress because he considered them to be inflationary and this year he threatened to veto an earlier version of the bill for the same reason.

Congress has now worked out a formula for the HEW budget which President Nixon still believes to be excessive but which he found politically acceptable. Essentially, Congress added some \$1,100 million to the Administration's budget request but it has also given President Nixon power to withhold up to \$400 million of the appropriate funds. An important aspect of the bill is that Nixon can lop no more than 5% off the funds allocated to any given programme. Thus congressionally determined priorities cannot be upset by the White House.

As far as the National Institutes of Health are concerned, even assuming that President Nixon exercises his authority to reduce the total HEW budget by \$400 million (as he almost

certainly will), every institute is set for at least a moderate budget increase. The National Cancer Institute will get \$523.6 million—an increase of \$23.6 million over the Administration's budget request—and the National Heart and Lung Institute will receive \$287.8 million. The largest increase, however, will go to the National Institute of General Medical Sciences, which is set to receive at least \$167.9 million; the Administration had wanted to spend only \$138.5 million. The National Institute of General Medical Sciences is primarily responsible for supporting basic research and Congress decided to give it a considerable increase after a string of influential scientists had complained that the crusades against cancer and heart and lung diseases were taking money away from important areas of basic research.

Research cuts irksome but tolerable

John Hall

ALTHOUGH cuts in British government spending will cause reductions of more than £8 million (5.5%) in research council budgets for next year, there is a feeling that the economies required are irksome rather than seriously debilitating for the five councils involved.

The Department of Education and Science, which was to have spent £151.6 million (at 1973 values) on research and postgraduate training through the research councils during 1974–75, has

issued a revised estimate of £143.5 million, following the chancellor's power crisis budget of December 17, when an overall reduction in public spending of £1,200 million was announced.

The Science Research Council (SRC), which was to have received £72.4 million, will now be given £2.6 million less; the Medical Research Council (MRC), due to have received £29.5 million, loses £1.3 million; the Agricultural Research Council's £23.9 million is cut by £1.4 million; the Natural Environment Research Council (NERC) loses £1 million from its projected £19.5 million; and the Social Science Research Council drops £1.8 million from a proposed £6.5 million.

The NERC is postponing work on two capital expenditure programmes (the Institute for Marine Environmental Research at Plymouth and the British Antarctic Survey building at Cambridge) but otherwise the councils are talking in terms of careful, across the board economies rather than of halting projects which are already under way.

The Agricultural Research Council gave an assurance that salaries and wages of existing staff would not be affected by the 16.3% cut it has to make. The reduction will be spread almost equally across capital and current expenditures, and the effect of the budget changes on individual research units has yet to be determined.

The NERC's saving will be of £600,000 on current and £400,000 on capital spending, and will be achieved by a process of "slowing things down

rather than actually cutting anything out".

An MRC spokesman's comment on the cuts was that £1.3 million might look like a lot of money but spread across its own 80 research establishments, and other users, it did not exactly amount to a crippling threat. In the same vein the SSRC promised that "such a small amount" could be saved by marginal reductions in the funds that were made available for new commitments in research and postgraduate training.

The SRC was keeping quiet about the cuts until its members could meet for discussions.

Knights Bachelor

In the New Year Honours List the following have been created Knights Bachelor:

Stanley George Hooker, Group Technical Director of Rolls-Royce (1971) Ltd;

Maurice George Kendall, for services to the theory of statistics;

John Cowdery Kendrew, for services to the Ministry of Defence;

Charles William Oatley, Emeritus Professor of Electrical Engineering, Cambridge University;

Hugh Norwood Robson, Vice-Chancellor, Sheffield University;

Frederick Henry Stewart, Regius Professor of Geology, Edinburgh University.

correspondence

Ethiopian Calamity

SIR,—In the article 'The Ethiopian Calamity' (*Nature*, 246, 53; 1973) your correspondent writes that a plea went to the World Food Programme but that "aid did not materialise from this source for several months".

After almost 11 years of food aid activities during which time this Programme has shipped some four million tons of food aid for social and economic development and emergencies, we have not found it possible to procure and deliver large quantities of food from ports in North America or Western Europe to developing regions in a matter of days or even weeks. This difficulty was fully appreciated by the Government of Ethiopia which, in its request to WFP for 20,000 tons of grain in April this year, asked for delivery by December

1973. Supplies are now arriving as planned. However, because of assurances of the arrival of WFP food, the Government was able to borrow from its national stocks and began distribution in May. The WFP food now arriving will be used to replenish these stocks.

Meanwhile, the Programme has arranged for shipment of a further 15,000 tons of grains plus an additional \$350,000 for internal transportation of relief supplies regardless of their source. This will bring WFP's total contribution to 25,000 tons of food grains and \$550,000 in cash.

Yours faithfully,
FRANCISCO AQUINO

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Fortuitous Correlations?

SIR,—The article 'What's in a Name?' (*Nature*, 246, 385; 1973) pointed out a number of significant correlations but ignored the rich contributions made by brain research workers to this field¹⁻⁴.

Yours faithfully,

G. CURZON

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¹ Looney, J. M. (A Biochemical Study of the Blood in Mental Disorders), *Am. J. Psychiat.*, 4, 29 (1924).

² Brain, W. R. (Mind and Matter), *Lancet*, 864 (1951).

³ Brain, W. R. (Henry Head: The Man and His Ideas), *Brain*, 84, 529 (1961).

⁴ Boring, E. G. (A History of Introspection), *Psychol. Bull.*, 50, 169 (1953).

news and views

Narcotic action at the molecular level

OPIUM and its derivatives have been used for centuries to ease the pain of injury and illness, and to blur the mental anguish of society's less fortunate. Morphine, the primary active ingredient of opium, has long been the standard analgetic. Morphine's medical usage, however, is constrained by the body's tolerance to opiates. Ever increasing dosage is required to maintain the analgetic effect. With chronic usage the body becomes dependent on the presence of opiate, and withdrawal causes agony.

For several decades scientists have sought a morphine analogue which would produce analgesia without tolerance (see Eddy and May's review in *Science*, **181**, 407; 1973). Attempts to elucidate the mechanism of opiate action have been spurred on by the epidemic of heroin (diacetyl-morphine) abuse which has become a socio-medical problem of staggering proportions in most western cities.

Last spring Pert and Snyder announced their biochemical demonstration of a receptor in nervous tissue which binds opiates with stereospecific selectivity and with affinity paralleling pharmacological potency (*Science*, **179**, 1011; 1973). In the ensuing months a stream of reports from Snyder's laboratory has demonstrated the utility of their assay, both for studying the basic pharmacology of opiate action and for rapid screening of narcotics with medical potential.

In *Nature* on October 26, Kuhar, Pert and Snyder (**245**, 447; 1973) described detailed mapping of the concentration of opiate binding sites in both human and monkey brains. They discovered striking regional variation in binding, with receptor concentrations varying more than thirty-fold between the highest and the lowest areas. Interestingly, the regions high in opiate binding all lie within the limbic system. Binding is greatest within the anterior amygdala, uniformly high throughout the hypothalamus, and high in medial but not in lateral thalamus. Frontal cortex areas show moderate binding of opiate, but little is found elsewhere in the cerebrum. This distribution leads to intriguing speculation about how the analgetic and euphoric effects of opiates might be related to limbic involvement in perception and expression of emotion and in the direction of motivated behaviour.

How well does the density of opiate binding sites correspond to the locations at which externally administered narcotics affect the brain? Previous workers have inserted morphine into various brain regions of living animals and tested for analgetic response; the distributions of opiate binding and of pharmacological effect seem well correlated.

There has been considerable controversy over whether opiate action is associated with some particular neurotransmitter. From the evidence various proponents have presented, one may conclude that the target of opiates is serotonergic, or noradrenergic, or perhaps cholinergic. In this study, Snyder's group asked whether opiate binding could be correlated with distributions of acetylcholine, catecholamines, GABA or serotonin. Disappointingly, although there were some similarities, there was no clear-cut parallel in any case. Furthermore, lesions of well known nerve tracts specific for acetylcholine, noradrenaline, or serotonin produced no change in opiate binding in the affected areas.

Much narcotic research has centred on searches for the

basis of the contrasting actions of opiate agonists and opiate antagonists. Many modifications of the morphine chemical structure have been studied in search of an analogue with a more amenable spectrum of pharmacological activity. In the process of searching, there were discovered certain analogues which block completely the action of opiate agonists (structures with morphine-like action). These 'antagonists' do not cause dependency, and in fact dramatically precipitate withdrawal in morphine-dependent animals. Safe, long lasting opiate antagonists are avidly being sought, with the idea of 'treating' addicts by thwarting the effect of heroin consumption.

Pert and Snyder have recently shown that the various opiate agonists and antagonists have a wide range of binding affinities for nervous tissue, and that there is a high correlation between affinity and pharmacological efficacy of these drugs *in vivo* (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 2243; 1973). Agonists and antagonists apparently compete for the same receptors, and because there is complete overlap in the range of affinities of the two classes of opiate, it would seem that the difference in their pharmacological effect must lie in some difference in cellular response ('intrinsic action') due to contrasting structure. Pert, Pasternak and Snyder have now discovered a difference in the way agonists and antagonists are bound by brain tissue, and that this differential effect can be used to discriminate *in vitro* between the two classes of opiate (*Science*, **182**, 1359; 1973). They first found that injection of mice with either opiate agonists or antagonists produced within minutes a significant increase in the number of opiate binding sites. The narcotic antagonists were 10 to 1,000 times more potent than structurally related agonists in enhancing receptor binding, which corresponds well to the fact that antagonists affect the body at much lower concentrations than do agonists.

In the light of the far greater pharmacological potency of narcotic antagonists, it seemed puzzling that agonists, such as morphine, oxymorphone and levorphanol, showed *in vitro* binding affinities quite similar to those of the structurally corresponding antagonist derivatives nalorphine, naloxone and levallorphan (respectively). This puzzle was resolved by the propitious discovery that adding sodium to the assay medium (in concentrations corresponding to those found in the body) considerably increased antagonist binding, while lowering that of agonists. Interestingly, the increase in binding is caused not by a change in receptor affinity but by an increase in the number of receptor sites available.

The discovery of this differential ionic effect on binding offers the prospect of a simple biochemical test for discriminating the agonist and antagonist activities of drugs which might be used for treatment of heroin addiction or which might provide analgesia without physical dependency. The authors examined the ability of a series of known opiate agonists and antagonists to inhibit the binding of tritium-labelled naloxone (an antagonist), both in conditions of low sodium and of high sodium. High sodium did not alter the binding of antagonists, but drastically lowered the ability of agonists to compete for receptor sites. Pentazocine, which has a mixture of agonist and antagonist properties, showed an intermediate loss of binding. Such mixed action drugs are clinically important because they offer the possibility of 'non-addicting' analgesia. It is theorised that the ability to block pain is a function of agonist affinity, and that addiction is prevented when agonist and antagonist actions are present simultaneously.

K. D.

Histone mRNA and maternal message

from a Correspondent

HISTONE mRNA not surprisingly may be isolated from many cell types as a 7-9S component which resolves on acrylamide gels into six or seven bands (Weinberg *et al.*, *Nature*, **240**, 225; 1972). Until recently the evidence in favour of these RNAs being truly histone messages was circumstantial, but Jacobs-Lorena, Baglioni and Borum (*Proc. natn. Acad. Sci. U.S.A.*, **69**, 2095; 1972) have provided proof that a 7-9S RNA species is translated into histones in an ascites cell free system. The original work of Robbins and Borun (*Proc. natn. Acad. Sci. U.S.A.*, **57**, 409; 1967) showed that in HeLa cells histones are synthesised during S phase on small cytoplasmic polyribosomes and this led to the identification of a rapidly labelled 9S RNA species associated with them (Borum, Scharff and Robins, *Proc. natn. Acad. Sci. U.S.A.*, **58**, 1977; 1967).

Early work by Kedes and Gross on sea urchin embryos (*Nature*, **223**, 1335; 1969; and *J. molec. Biol.*, **42**, 559; 1969) confirmed the existence of a newly synthesised 9S RNA species associated with small polysomes during the phase of rapid cell division called cleavage at the start of development. The system was notable for the very high specific activity to which the 9S RNA could be labelled, for the apparent ease of 9S RNA purification and for its freedom from contamination by labelled ribosomal RNA, which is not synthesised in appreciable quantities until much later. It is not difficult to think of things to do with highly labelled mRNAs even though they lack authentic credentials and, first, Kedes and Birnstiel (*Nature new Biol.*, **230**, 165; 1971), in what they regarded as the first steps to the isolation of a eukaryotic gene coding for a specific protein, produced evidence for reiterated, closely clustered histone coding sequences potentially separable from the rest of the genome.

Weinberg, Birnstiel, Purdom and Williamson (*Nature*, **240**, 225; 1972) took this analysis further by showing that component species separable from 9S histone messages each hybridise to DNA sequences several hundred times repetitive. The different groups of repetitive histone DNA seem to be closely linked since hybridisation of the RNA subfractions to DNA banded in CsCl shows that they all fall in the same region at a density (1.705 g cc^{-1}) just greater than the main band (1.697 g cc^{-1}).

In spite of the fact that histone 9S mRNA synthesis starts soon after fertilisation in sea urchins, treatment with actinomycin D at concentrations which inhibit synthesis permits many

cleavages and apparently normal development to the blastula stage (Gross and Cousineau, *Exptl cell Res.*, **37**, 368; 1964). Embryos thus treated continue to synthesise proteins though not nuclear proteins during this time (Raff, Greenhouse, Gross and Gross, *J. cell Biol.*, **50**, 520; 1971) and this was regarded as indirect evidence for the absence of stored histone message in unfertilised eggs.

Two recent papers from Gross and his colleagues now provide direct proof that histone mRNA on the contrary is stored in unfertilised sea urchin eggs although at a lower level than that attained during cleavage, and this constitutes the first demonstration of a maternally-inherited message with a defined coding sequence. Many previous attempts have been made to provide direct evidence for stored maternal mRNA in echinoderm (Slater and Spiegelman, *Proc. natn. Acad. Sci. U.S.A.*, **56**, 164; 1966) and *Xenopus* (Davidson, Crippa, Kramer and Mirsky, *ibid.*, **66**, 379; 1972) embryos, but in none of these cases was an identifiable maternal RNA species shown to stimulate the synthesis of a defined product.

What Gross *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2614; 1973) did was to extract RNA from different regions of a sucrose gradient in which a post mitochondrial supernatant from homogenised unfertilised echinoderm eggs had been centrifuged such that the 80S ribosomes were almost pelleted. They found that a mouse ascites cell free system will make protein from these RNA preparations but that the most active fractions are from the ribosome subunit region of the gradient (20-40S). Using aspartic acid and lysine to show up differences in mRNA coding sequence they found that the faster sedimenting RNP fractions give RNA which incorporates more aspartic acid relative to lysine than the more slowly sedimenting fractions. By running the labelled products in acrylamide gels together with authentic labelled histone, and by analysing tryptic peptides, they were able to show that the RNA from a slowly sedimenting (20S) RNP fraction makes histone protein almost exclusively and that the more rapidly an RNP sediments beyond 20S the less histone protein will be made by its RNA. These experiments show that mRNAs exist in non-ribosome bound RNP particles from unfertilised sea urchin eggs and that they are capable of stimulating the synthesis of histone in a cell free protein synthesising system.

Skoultschi and Gross (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 2840; 1973), using the completely different approach of competition-hybridisation to detect specific mRNA sequences, have been able to provide direct support for the conclusions drawn from *in vitro* protein

synthesis studies. They used ^3H -labelled 9S RNA from sea urchin embryos in conditions in which most of the input labelled RNA can form hybrids with DNA but which still allows identical unlabelled RNA to compete for sites. Both total RNA from small polysomes and unfertilised eggs competed in the reactions but purified 18 and 26S ribosomal RNA, rabbit globin mRNA and 9S histone mRNA from another echinoderm species did not. The curves generated suggest that the amount of histone mRNA in unfertilised eggs is one-quarter of that in the small polysomes of an embryo of 100 cells. This figure is in agreement with similar work by Farquhar and McCarthy (*Biochem. biophys. Res. Comm.*, **53**, 515; 1973), who used very different conditions for their hybridisation.

Skoultschi and Gross went on to show that RNA from subribosomal RNP particles from unfertilised eggs competes with 9S histone mRNA in agreement with the prediction from the protein synthesis studies of Gross *et al.* These papers represent a real advance since they unequivocally identify by two very different methods a messenger RNA which is maternally inherited.

Role of plastocyanin in photosynthesis

from our Photosynthesis Correspondent

THE old argument whether or not plastocyanin acts between cytochrome *f* and the reaction centre of photosystem 1 (P700) now seems to be over. It has been known for many years that this copper containing protein is an important component of the photosynthetic electron transport chain but there have been several contradictory hypotheses about its exact site of action. The most widely accepted view is that plastocyanin acts as an intermediate between cyt *f* and P700. Not all investigators, however, agree with this scheme and prefer to place plastocyanin on the oxidising side of cyt *f* so that cyt *f* acts as the primary electron donor to P700. Alternative suggestions have been that plastocyanin and cyt *f* act in parallel in such a way that they both donate electrons to P700 or that they are associated with the different photosystems; plastocyanin with photosystem 2 and cyt *f* with photosystem 1.

It now seems from a recent publication from San Pietro's laboratory at Indiana (Siedow, Curtis and San Pietro, *Arch. Biochem. Biophys.*, **158**, 889; 1973) that the most popular hypothesis is correct. In a very careful piece of work Siedow *et al.* have demonstrated that plastocyanin acts stoichiometrically as an electron carrier between cyt *f* and P700. Their work is particularly convincing because they have managed to

prepare subchloroplast particles which are completely depleted of plastocyanin. These particles were obtained by sonicating osmotically shocked chloroplasts suspended in a medium containing a low concentration of pyridine. Using difference absorption spectroscopy Siedow *et al.* have demonstrated that these particles were unable to support the photo-oxidation of cyt *f* until plastocyanin was added to the suspension. Moreover, they found that it was necessary to either treat the particles with non-ionic detergents such as digitonin or sonicate them after adding the plastocyanin to induce full photo-oxidation of the cytochrome.

The results of these experiments clearly support a strict linear sequence of electron transport on the oxidising side of photosystem I from cyt *f* → plastocyanin → P700. Furthermore, the requirement for sonication or detergent treatment to bring about the full reconstitution supports a concept already put forward by Hauska *et al.* (*J. biol. Chem.*, **246**, 189; 1971) that plastocyanin, *in situ*, binds to a specific site on the inner surface of the chloroplast membrane.

Structural analysis by electron diffraction

from a Correspondent

A JOINT meeting of the Crystallography and Electron Microscopy Groups of the Institute of Physics was held at the Institute of Electrical Engineers, London, on November 15–16 to discuss the use of electron diffraction for structure determination. The contributions covered electron diffraction from gases and surfaces, structural analysis of thin films by combined electron diffraction and microscopy, direct lattice resolution by electron microscopy, as well as applications of these techniques to the study of molecular crystals and minerals. There was also a discussion of the interpretation of electron micrographs and diffraction patterns of amorphous materials.

Gases and surfaces

D. W. J. Cruickshank (UMIST, Manchester) opened the meeting with a review of electron diffraction from gases. Since the 1930s some twenty groups around the world have analysed between them more than 1,000 molecules. The techniques and methods of interpretation are by now fairly standard and the interest lies in the chemistry of the molecules themselves. It is particularly interesting to compare rather similar molecules and to find surprises—for example, $(\text{SiH}_3)_3\text{N}$ is a planar molecule whereas $(\text{SiH}_3)_3\text{P}$ is pyramidal. Similarly, in the so-called 'sandwich' molecules $(\text{C}_6\text{H}_6)_2\text{Cr}$ and

$(\text{C}_5\text{H}_5)_2\text{Fe}$, the metal atoms are symmetrically placed between the rings but in $(\text{C}_5\text{H}_5)_2\text{Be}$ the Be atom is asymmetrically placed and in $(\text{C}_3\text{H}_3)_2\text{Ni}$ there are two variants, in one of which the two carbon frameworks are not parallel.

M. Prutton (University of York) gave an account of the determination of surface structures by RHEED (reflection high energy electron diffraction) and LEED (low energy electron diffraction). Both of these methods are sensitive to the crystallography of the surface layers because of the low penetration of the electron beam and both can be used to obtain the mesh sizes of the surface and of adsorbed layers. The use of quantitative intensity data to deduce the position of atoms in the unit mesh has, however, proved more difficult. In the case of RHEED, transmission diffraction through asperities presents an experimental problem and surfaces with small roughness on the 10 Å scale are needed. For LEED, although progress in the theory has been rapid recently, Prutton felt that it was still extremely difficult to make good quantitative statements. This point was (perhaps inadvertently) reinforced by M. A. van Hove (University of Cambridge), who indicated that in the case of oxygen adsorbed on (100) Ni in a $c(2 \times 2)$ structure, the oxygen atom has been deduced by different workers to be either 1.5 ± 0.1 Å or 0.9 ± 0.1 Å above the Ni atoms, with an almost equally good fit to theory in both cases, but that values in between were impossible on the basis of a match between dynamical LEED theory and experiment. Of the two possible values the first is much more plausible from a chemical viewpoint.

The information obtainable from an understanding of the dynamical transmission diffraction effects through thin films was reviewed by J. W. Steeds (University of Bristol). Of the two techniques considered, the convergent beam technique and the analysis of electron microscope images of bent crystals, it is the second which is easier to use for qualitative crystal structure determination. Steeds showed that one could easily use the form of bend contours to deduce the point group of the crystal and that dark-field observation could establish the presence of centres of symmetry, glide planes and screw axes, which could lead to the establishment of the space group. Applications to layer structures such as mica and molybdenite were described in this and in the following papers by G. J. Tatlock and Steeds. The simulation of these bend contours using many-beam dynamical theory yielded impressive agreement with the experimental contours, and enabled them to distinguish easily different polytypes of molybdenite and to detect the presence of otherwise invisible stacking faults.

Lattice images

J. Hutchison (University of Oxford) gave a fascinating glimpse into the future with his talk on direct lattice resolution by electron microscopy. In the past few years the electron microscope has been used to study the lattice images of several structures including a class of complex oxides called block structures based on NbO_6 octahedra. The octahedra are stacked together in blocks of, say, 4×3 or 5×3 and have tetrahedral sites at the corner, where four blocks join together, which can accommodate substitutional metal ions. In the extremely high resolution micrographs shown, the blocks and the positions of the heavy atoms in a compound such as $\text{W}_4\text{Nb}_{26}\text{O}_{77}$ were clearly seen, and the experimental images were in excellent agreement with simulated images based on *n*-beam dynamical theory; one can almost resolve the individual octahedra in both the experimental and simulated images. The images depend in detail on the defocus of the objective lens, however, so that it is essential to know the amount of defocus and include this in the calculations.

Quasi-amorphous films

The use of dynamical diffraction theory for transmission electron diffraction from single crystal films is well established and Hutchison and Steeds elegantly showed how well the theory works. By contrast, the best theory for describing the diffraction from quasi-amorphous films is not nearly so clear; this was the theme of a panel discussion between W. Cochran (University of Edinburgh), A. Howie (University of Cambridge) and M. V. Berry (University of Bristol) under the chairmanship of D. J. H. Cockayne (University of Oxford). The problem arises because there are many possible structures of quasi-amorphous solids (such as amorphous Si and Ge) which would give the same diffraction pattern. Can lattice imaging in the electron microscope be used to give extra information? Previous work by Rudee and Howie had suggested the existence of microcrystallites, some 15 Å in diameter, on the basis of groups of 'lattice' fringes observed using a particular part of the diffraction pattern to form the image. These small regions are, however, embedded inside a film of thickness ≈ 200 Å, so that they are certainly not isolated crystallites, and the interference with the regions above and below any such crystallites, as well as the degree of defocus, are important in determining the image. Cochran has performed simulations of the image of a random network model and considers that although the fringes are unlikely to be due to such a structure, they do not really require a microcrystallite model

either. He showed that changes in defocus during the exposure could cause certain periodicities in the image to be enhanced. Berry was inclined to favour an approach based on simple image transfer theory which he claimed means that the fringes in the image were merely random noise, spatially filtered by the objective aperture; provided a relatively small segment of the diffraction ring is used to form the image, groups of fringes would be observed, essentially independent of the detailed structure of the sample. Thus it is clear that the unravelling of the detailed structure of these quasi-amorphous films remains a difficult problem which is far from solved, though it is certain that electron microscopy has a part to play in its resolution.

Molecular solids

The application of electron microscopy and diffraction to the study of molecular solids and minerals was the subject of the last session at the meeting. C. A. English (University of Sussex) reviewed the work on solid rare gases, O_2 , N_2 and CO_2 , which has been carried out largely at the University of Sussex. An electron microscope fitted with a liquid helium stage is used and single crystals can be produced by annealing the thin films obtained by vapour deposition at a pressure of a few torr. Among the examples he described were the determination of the structure of α - N_2 , the measurement of the stacking fault energy of solid xenon, twinning in β - O_2 and radiation damage in solid O_2 , N_2 and CO_2 .

An elegant structure determination of a molecular solid, poly sulphur nitride, by electron diffraction, was described by M. Boudeulle and P. Michel (University of Lyon). Also, the complexities of graphite-halogen intercalation compounds, including structures with very long period order giving rise to the very beautiful diffraction patterns, shown by W. T. Eeles (UKAEA, Capenhurst), revealed that there is much to be learned about the bonding in all the molecular solids discussed. The advantages of combined use of electron microscopy and single crystal electron diffraction when the crystals are too small for single crystal X-ray work were well illustrated in all these contributions.

Minerals

This same theme was reinforced by P. E. Champness (University of Manchester) in her review of electron diffraction and microscopy as a means of characterising minerals. Analysis of the characteristic X rays by EMMA (combined electron microscopy and microprobe analysis) to obtain chemical information from regions as small as

1,000 Å in diameter is also invaluable. A particularly impressive example of the combined use of these techniques is the study of an igneous pyroxene containing lamellae around 0.1 µm thick spaced about 50 to 100 µm apart. In between the lamellae there are very fine precipitates with a precipitate free zone next to the lamellae. The orientation relationships of all the phases can be determined by electron diffraction. Analysis of the Ca/Si X-ray intensity ratio reveals that the lamellae are rich in calcium and the diffusion profile of the calcium content between adjacent lamellae can thus be determined. The fine precipitates can be imaged by lattice resolution of the 1.8 nm spacing and shown to be only one unit cell thick.

The conference clearly showed the range of opportunities offered by electron diffraction, particularly when it is used in combination with electron microscopy and other techniques such as X-ray analysis. The greater difficulty of interpretation *vis a vis* X-ray diffraction remains, but well-founded dynamical diffraction theory is being used with very considerable success for quantitative work on thin films with high energy electrons. At low energy, theoretical improvements are rapidly being made, but much remains to be done before the theory can be used with complete confidence. Direct structure determination by high resolution electron microscopy is now a fascinating possibility and will undoubtedly be explored further in the future.

If M haemoglobin, then R or T?

from a Correspondent

MUCH ingenuity has been shown in utilising the structural differences found in the genetically determined haemoglobin variants to assist the study of the relationships between structure and function of normal haemoglobin. A striking example of this approach is the work of Mayer, Ogawa, Shulman and Gersonde (*J. molec. Biol.*, **80**, 187; 1973) on high-resolution proton nuclear magnetic resonance (NMR) spectral studies of the quaternary state of haemoglobin M(Iwate).

Hb-M(Iwate) is an α -chain variant ($\alpha^{88}\text{His} \rightarrow \text{Tyr}$, β_2) and is identical with Hb-M(Oldenburg) and Hb-M(Kanakee). Apart from shortening the list of M-type haemoglobins, the locations in which it has been discovered provide an interesting example of the probable independent occurrence of the same mutation in three widely separated places. As with other M-type haemoglobins the chemical properties of the haems on the variant chains are abnormal. It has previously been shown that in the crystal the quaternary state

is deoxy (T) when the normal β -chain subunits are deoxygenated and also when they are oxidised to methaemoglobin and therefore ligated with H_2O . In view of the low oxygen affinity of the variant it seemed probable that after ligation it remains in the T quaternary structure in solution as well as in the crystal. Mayer and colleagues have therefore used Hb-M(Iwate) to test the extent to which three NMR spectral features are linked to the state of the quaternary structure.

These three features are (1) the paramagnetically-shifted resonances of the cyanide-ligated subunits in valency-hybrid haemoglobin, (2) the upfield resonance in oxy and carbonmonoxy-haemoglobin assigned to Val E11 near the haem group, and (3) the low-field resonance near -14 p.p.m. (referred to 2,2-dimethyl-2-silapentane-5-sulphonate) in deoxyhaemoglobin which comes from an exchangeable proton, and which disappears on ligation. Although these three features had been used to distinguish between oxy (R) and deoxy (T) quaternary structures, their validity had not been tested with a haemoglobin known to be in the T quaternary state in solution even when ligated.

A comparison of the 220 MHz NMR spectra of normal human adult haemoglobin valency hybrids and of Hb-M(Iwate) in the presence and absence of organic phosphates clearly shows that those NMR spectral features previously attributed to the deoxy (T) quaternary structure are present in Hb-M(Iwate) when the β subunits are ligated to O_2 , CO or CN^- as well as when they are unligated, with the quaternary structure remaining in the deoxy (T) state throughout. These results complement previous spectral studies on Hb-Kansas ($\alpha_2\beta_2$ 102 Asn $\rightarrow$ Thr) and on valency-hybrid haemoglobin.

In the authors' view, the increasing body of evidence that the quaternary structure is the most important factor in determining the functional properties of haemoglobin provides additional support for a two-state model, as proposed by Monod, Wyman and Changeux in 1965, as a suitable first-order description of haemoglobin function.

In another application of NMR spectroscopy to the study of haemoglobin, Manuck, Maloney and Sykes (*ibid.*, 199) have studied the binding of methyl isonitrile to the β chains by measuring the transverse relaxation times (T_2) of the isonitrile protons as a function of protein concentration, temperature and ^{15}N decoupling. Binding both at the haem iron and at non-specific site(s) affects the measured relaxation times. From the results a value of 57 ± 12 s $^{-1}$ for the 'off' rate constant (k_{-1}) for specific binding, and an Arrhenius activation energy for k_{-1} of 14 ± 3 kcal mol $^{-1}$, were obtained.

Erasmus Darwin, master of many crafts

Desmond King-Hele

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Erasmus Darwin was famous in the eighteenth century as a physician, an inventor and a best-selling poet. Today he earns most credit for recognising biological evolution, discovering how clouds form, analysing plant nutrition and influencing the English Romantic poets.

DR ERASMUS DARWIN, who lived from 1731 until 1802, achieved more in a greater variety of intellectual disciplines than anyone since. Why then has he not gained the fame he deserves? Partly, perhaps, because we feel that his family already has enough fame, through his grandson Charles; and partly because of our unspoken but deep conviction that no one can really master many different crafts, as Darwin did. He belongs with Leonardo da Vinci and Goethe, immensely capable in practice and theory, in life, in literature and in science.

By profession Darwin was a physician, generally recognised as the finest doctor of his time in England. "Within the narrow resources of 18th-century medicine, he was supreme"¹, but he refused King George III's repeated requests to become royal physician². By nature Darwin was a large and powerful-looking man (Fig. 1), cheerful and healthy, and, despite a stammer, a witty and rather domineering talker. By inclination he was extremely sociable and inventive. With Matthew Boulton and William Small, he founded the Lunar Society of Birmingham³, which was the real intellectual driving force behind the Industrial Revolution in England and hence the modern technological world⁴. Encouraged by his Lunar friends, especially Watt and Wedgwood, Darwin produced working inventions ranging from a speaking machine to a horizontal windmill, and dozens of designs on paper, from a canal lift to a steam turbine. Darwin won greatest acclaim, however, in a quite different sphere, as a poet: his long poem *The Botanic Garden* took the literary world by storm in the early 1790s. Coleridge called him "the first literary character in Europe"⁵, the Napoleon of literature, as it were.

Today Darwin earns most credit among scientists for his recognition of biological evolution^{6,7}, his psychological ideas⁸, his analysis of plant nutrition and photosynthesis⁹, and his discovery of the main process of cloud formation¹⁰. And the literary critics are ready to honour him not for his poems but for his immense influence over the English Romantic poets, Wordsworth^{11,12}, Coleridge¹¹⁻¹⁶, Shelley^{11,17} and Keats^{18,19}.

Lichfield and the Lunar Society

Erasmus Darwin was born at Elston near Nottingham, the third son of a moderately successful lawyer. He went to Chesterfield School and from there to Cambridge, where he studied medicine and took his BA in 1754. Then came two years at the leading medical school, at Edinburgh. After this thorough schooling in dubious medical theory, he was ready to be loosed on the public. Finding no patients at Nottingham, he migrated to Lichfield, where he had the good fortune—or perhaps skill?—to cure a prominent local man whom all other doctors had condemned to death²⁰. His future career assured

by this *coup de théâtre*, Darwin settled at Lichfield. From 1758 until 1781, living in the pleasant house which still stands on Beacon Street at the western extremity of the Cathedral Close, he gave medical advice expensively to the rich and freely to the poor.

Today Lichfield is overshadowed by its neighbour Birmingham 10 miles to the south; but in the eighteenth century their roles were more nearly equal, and the Cathedral Close housed a literary coterie, headed by Canon Seward, the Dean, who edited the plays of Beaumont and Fletcher, as if to rival Lichfield's leading literary figure, Dr Johnson. As Darwin put it,

From Lichfield famed two giant critics come,
Tremble, ye poets! hear them! 'Fe, Fo, Fum!'
By Seward's arm the mangled Beaumont bled,
And Johnson grinds poor Shakespeare's bones for bread²².

Canon Seward's daughter Anna, who was 14 when Darwin arrived at Lichfield, later became quite famous as a poet, with some help from Darwin: she was known as "the Swan of Lichfield" in insulting analogy with "the Swan of Avon".

In 1758 Darwin married Mary Howard, whose family also lived in the Close; the marriage proved a very happy one, marred only by Mary's continual ill-health. They had three sons who survived infancy. The eldest, Charles, was a brilliant medical student, but he died tragically at the age of 19 from an infection, after cutting his hand while dissecting. The second son, Erasmus, became a lawyer. The third, Robert, followed in his father's footsteps, becoming a successful doctor at Shrewsbury; he was an impressive man, not only in appearance (he weighed 24 stones) but also in ability, and especially in his uncanny power of reading character, much admired by his son Charles²³.

Erasmus Darwin was at his most inventive and socially active during the 1760s. One of his earliest friends was Matthew Boulton^{24,25}, then a humble manufacturer of buckles in nearby Birmingham. But his energy and enterprise were to make Boulton the chief manufacturer of England and "the father of Birmingham". Darwin's "favourite friend" in the late 1760s was the learned and liberal Dr William Small, recently returned from teaching in America, where Thomas Jefferson, one of his pupils, declared that Small's example "fixed the destinies of my life". About 1766 Darwin, Boulton and Small began those monthly meetings of friends at the time of the full Moon, which were soon to develop into what became known as the Lunar Society of Birmingham.

In the early days of his friendship with Boulton, in 1763, Darwin was madly enthusiastic about the idea of a steam carriage with twin cylinders, which he hoped Boulton would help him to construct. Boulton was rightly cautious, but Darwin's enthusiasm for steam was not wasted: James Watt on his first visit to England in 1767 called on Darwin^{25,26} and began a lasting friendship, which benefited both. Watt was rather gloomy by nature and needed encouragement: who better to provide it than the energetic and optimistic steam-enthusiast Darwin? No wonder Watt, though usually reticent,



Fig. 1 Erasmus Darwin from a portrait-bust by William Coffee, reproduced by courtesy of Sir Robin Darwin.

praised Darwin so highly: "It will be my pride while I live that I have enjoyed the friendship of such a man"²⁷.

Another man who enjoyed that friendship to the full was Josiah Wedgwood²⁸, who met Darwin when both were about 30. When in 1765 Wedgwood and Brindley began promoting what was virtually a new transport system, the Trent-and-Mersey canal, Darwin was an enthusiastic supporter. Darwin's horizontal windmill was used for grinding colours at Wedgwood's factory for 14 years, until displaced by steam. The Darwin-Wedgwood alliance was strengthened by the marriage of Robert Darwin with Susannah Wedgwood in 1796.

One of Darwin's friends at Edinburgh had been the chemist James Keir²⁹, who after a spell in the Army settled at West Bromwich in 1768, and later set up an alkali factory at Tipton nearby. Keir was a pioneer of the chemical industry, and his clever process of obtaining soda from salt in dilute solution³⁰ was commercially successful many years before the Leblanc process was devised.

On his daily rounds as a doctor, Darwin was bumped and joggled over the vile roads in his carriage, so it is not surprising that he was keen to construct comfortable carriages. Wedgwood greatly admired a design which used the wheel spokes as springs (an idea continually being resuscitated, even today³¹) with each spoke shaped in the serpentine line of beauty. A carriage of Darwin's, peculiarly resistant to overturning, greatly impressed Richard Edgeworth³², and inspired him to become an inventor. He visited Darwin in 1766 and became a lifelong friend.

This group of Darwin's friends—Boulton, Small, Watt, Wedgwood, Keir and Edgeworth—formed the nucleus of the early Lunar Society; Priestley was a later addition to their number. "Intellectually the most effective provincial group that has ever come together in England", C. D. Darlington has called them⁶. Individually they were among the most energetic and intellectually ablest men of the era, and their meetings, by some strange alchemy, channelled their talents fruitfully, producing a unique forward thrust in science and technology.

Middle and later life

During the 1760s Darwin's reputation as a doctor continued to grow, but he had no cure to offer his wife Mary, who died in 1770; her tributes to her husband were carefully recorded by Anna Seward²⁰. In the 1770s Darwin remained at Lichfield, unmarried; but he had two illegitimate daughters, known as the Misses Parker.

Then in 1778 he fell in love with Mrs Elizabeth Pole, the lively, young and beautiful wife of a leading Derbyshire landowner, Colonel Sacheverel Pole of Radburn Hall. At first Darwin could only woo her in verse²⁰, but in 1780 Colonel Pole conveniently died and Darwin, though rather ugly, fat and fifty, brushed aside more eligible rivals and married Mrs Pole in 1781. They moved to Radburn and later to Derby. This second marriage proved as happy as the first, and the seven children of the marriage acknowledged their good fortune in having been brought up in such a happy atmosphere. This quiet happiness seems to have lasted throughout the 20 outwardly uneventful years of Darwin's second marriage¹². He died in 1802 at the age of 70, of a heart attack.

A flowering of verse

Though outwardly unspectacular, these later years saw the publication of Darwin's five books, each epoch-making in its own way. First, in 1789–91, when he was already 60, came his encyclopaedic poem, *The Botanic Garden*, which gripped the literary world like a fever: "Dr Darwin has destroyed my admiration for any poetry but his own"³³, wrote Horace Walpole, a sentiment echoed by many another hard-boiled literary pundit. Part II of the poem, "The Loves of the Plants", had been published first, in 1789, because it was lighter—968 rhyming couplets about the sex life of plants, nicely personified and backed by masses of scientific notes. Part I, "The Economy of Vegetation", published in 1791, belies its title: it is really a compendium of scientific knowledge in 1,224 rhyming couplets, again with lengthy notes in support. The complete *Botanic Garden*, which was beautifully reprinted in facsimile by Scholar Press last year³⁴, runs to 550 quarto pages.

Was it wrong of those readers of the 1790s to admire *The Botanic Garden*? The poem is certainly rather rambling and unstructured; but Darwin's polished couplets have a lustre that time cannot tarnish, as generations of literary critics confirm (see, for example, refs 35 to 38). To this skill in verse Darwin added three other talents: 'a greater range of knowledge than any other man in Europe', as Coleridge put it³⁹; originality in proposing new theories and foreseeing new technology; and an amazing lightness of touch—he is forever laughing at himself. As an example of all three talents, here is his forecast of mechanically-propelled cars and aircraft:

Soon shall thy arm, Unconquer'd Steam! afar
Drag the slow barge, or drive the rapid car;
Or on wide-waving wings expanded bear
The flying-chariot through the fields of air.
Fair crews triumphant, leaning from above,
Shall wave their fluttering kerchiefs as they move;
Or warrior bands alarm the gaping crowd,
And armies shrink beneath the shadowy cloud⁴⁰.

When Darwin switches from technology to plants in Part II, comedy is still in the ascendant as he promises to tell us

What Beaux and Beauties crowd the gaudy groves,
And woo and win their vegetable Loves.

For example in *Lychnis*, where the males and females are on different plants, each female saucily peeps out when mature:

In gay undress displays her rival charms,
And calls her wondering lovers to her arms⁴¹.

Science so pleasantly presented was much to the taste of the leisured reading public. As L. T. C. Rolt has remarked, Darwin performed 'the astonishing feat of rendering in imaginative terms which any literate man could then appreciate the sum of human knowledge at that time. It was an achievement which was never repeated or even attempted⁴²'.

Medicine, evolution, young ladies and plants

In his later years Darwin's fame as a doctor became excessive. With a lifetime's experience and few new discoveries to outdate it, he was a legendary figure, whose every word was eagerly sought—a status scarcely credible today when the mystique of the doctor has largely evaporated. Although his medical theory was unsound, Darwin deserved his fame as a doctor because he was so keenly observant and could often guess correctly the future course of an illness; his cheerful attitude and his sympathy with the patient also had a good effect. To this must be added his long and deep thought about medicine, expressed in the second of his books, the two massive volumes of his treatise on animal life, *Zoonomia*, published in 1794–96. Dr Beddoes called it 'perhaps the most original work ever composed by mortal Man', which would 'place the author amongst the greatest of mankind⁴³'. We do not value the book so highly today because its classification of diseases has too many flaws. But we can still admire some of his bold experimental cures, his linking of body and mind, which has been influential in philosophy^{8,14}, and his pioneer work in treating mental illness humanely instead of brutally. Whatever our reservations now, *Zoonomia* had great influence in its day: it went into a second edition in 1796 and a third (revised) edition in 1801; provoked a 560-page critical commentary in 1798; 'was translated into German, French and Italian and was honoured by the Pope by being placed in the *Index Expurgatorius*', as Charles Darwin remarked⁴⁴.

Zoonomia is mainly medical, but it has one riveting chapter on biological evolution. As I have explained in a previous article in *Nature*⁷, Erasmus appreciated and described the main mechanisms of evolution, and made it part of his habitua. thinking for 20 years. In the England of the 1790s, reeling from the shock of the French Revolution, evolution was quite unacceptable: denying that God created species was near-treason; but Darwin was not sentenced to transportation, only to satire. In the *Anti-Jacobin* magazine in 1798 he was ruthlessly lampooned by Canning and others⁴⁵, while Coleridge condemned his evolutionary speculation as mere "Darwinizing".

Erasmus Darwin's correct advocacy of evolution does not detract from Charles Darwin's achievements. Rather it helps to explain Charles's success, by bringing out the strong tradition of evolutionary ideas in the family. For Charles's father Robert followed Erasmus in his profession and in his ideas (on alcohol and religion as well as evolution).

Erasmus carried his evolutionary ideas further than his grandson did, weaving it into his philosophy of life. Erasmus saw the full horror of the struggle for existence, more clearly than anyone else in his day, but he saw the bright side too. He believed most creatures delight in life; so, the better adapted they are, the happier they will be. Evolution is, in that sense, the survival of the happiest. Although highly organised animals have a greater capacity for happiness than lowly creatures, the plants and insects by their vast numbers make up a large part of the sum of happiness. This is the basis of his remarkable philosophy of organic happiness. He looks on rocks like limestone, chalk and coral, the remains of once-happy creatures, as "mighty monuments of past delight" and thinks we "should eye with tenderness all living forms". He is very much in favour of sexual love, not only because sexual generation speeded up evolution by increasing variety, but also because sex is a major component of organic happiness—he regards sexual pleasure as the greatest source of delight for lower creatures as well as human beings.

The third of Darwin's books is (for a change) a slim volume of 128 pages, entitled *A Plan for the Conduct of Female Education in Boarding Schools* (1797). Reprinted⁴⁶ in 1968, this is probably the most widely read of his books because of its significance in the history of education^{47,48}. Darwin totally opposed the conventional idea of his time that girls should become feather-brained simpering misses: he wanted to see them "sound in body and mind". "The advantages of a good education consist in uniting health and agility of body with cheerfulness and activity of mind"⁴⁶. His book, written for his daughters Susan and Mary Parker (who had set up a school), marks a quantum jump in the progress of girls' education, almost approaching modern ideas of sexual equality. Darwin deserves a small memorial in the pantheon of Women's Lib—though ironically the church where he was buried was burnt to the ground by suffragettes in 1914.

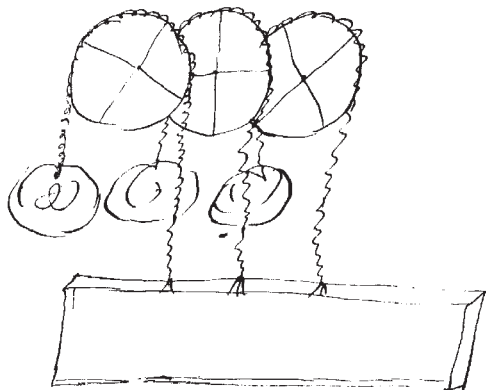
The fourth of Darwin's books, *Phytologia* (1800), is an impressive 600-page survey of vegetable life and all that sustains it, from carbon dioxide down to manures. *Phytologia* is a much better book than *Zoonomia* because it has so many ideas that are correct and new. One example is his discussion of plant nutrition, which is a great advance on any previous analysis. He specifies the process of photosynthesis, not of course in one neat equation ($\text{CO}_2 + \text{water} + \text{light energy} = \text{sugar} + \text{oxygen}$), but in separated sentences:

"This carbonic gas [CO_2] . . . is the principal food of plants. . . . Next to carbonic gas . . . water seems to afford the principal food of vegetables . . . because their solid fibres consist principally of carbon, and their fluids of water . . . When vegetable leaves are exposed to the sun's light they seem to give up oxygen gas. . . . Sugar is the principal nutriment of both animal and vegetable beings . . . the wonderful effect of vegetable digestion in producing sugar may be deduced from the great product of the sugar cane"⁴⁹.

Although Ingenhousz (1779) had shown that plants absorb

carbon dioxide and give out oxygen in sunlight, Darwin goes much further towards the complete reaction, which was elucidated by Liebig and others some 40 years later.

Darwin also discusses other elements essential to plant nutrition, and was the first to recognise the importance of nitrogen and phosphorus⁵⁰. He discusses nitrogen in detail, correctly specifying the role of ammonia and the formation of nitrates. He was well on the road towards defining the carbon and nitrogen cycles. Phosphorus he believed to be present in all plants because of their tendency to phosphoresce when decaying, and he recommends a search for phosphates as fertilisers—particularly calcium phosphate, since he also thought calcium was a vital element.



nothing is to be overcome but the int. inertia & friction, as the water box is always the same weight, whether the boat is in it or not, since the boat whether full or empty will displace just as much water as it's own weight.

Perhaps even the five argine beams, which are preferable to wheels? perhaps the best balance would be another trough full of water, as they might both of them be let dry if necessary occasionally.

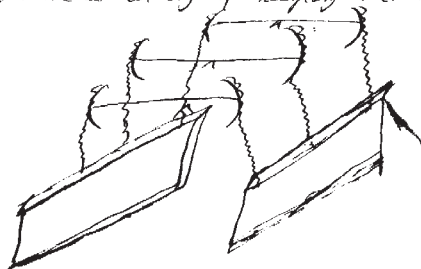


Fig. 2 Some of Darwin's sketches for a canal lift, from his *Commonplace Book* (1777). Reproduced by courtesy of The Royal College of Surgeons of England.

Among the many other bright ideas in *Phytologia* are sewage farms, biological control of insects, engineering drawings for new water pumps and drill ploughs, a seminal discussion of the individuality of buds, and proposals for a system of artesian wells⁵¹.

The pageant of life

The last of Darwin's five books is another long poem, *The Temple of Nature*, published posthumously in 1803. This is in my view his finest achievement, and its republication in facsimile by Scolar Press recently⁵² should bring it back into circulation after 150 years of unjust neglect. In *The Temple of Nature* Darwin traces the evolution of life from microscopic

specks in primaevae oceans through plant life, fishes, amphibia, land animals and birds:

Organic Life beneath the shoreless waves
Was born, and nurs'd in Ocean's pearly caves;
First forms minute, unseen by spheric glass,
Move on the mud, or pierce the watery mass;
These, as successive generations bloom,
New powers acquire, and larger limbs assume;
Whence countless groups of vegetation spring,
And breathing realms of fin, and feet, and wing⁵³.

The couplets are just as bright as in *The Botanic Garden* and Darwin manages to cram in even more information per line. But the really astonishing feature is the calm and correct description of the processes of evolution more than 50 years before the *Origin of Species* and 100 years before the details were generally agreed. For example, Darwin nonchalantly tells us about the transformation from fish through amphibia to reptiles. Some of the marine life, he says,

Leaves the cold caverns of the deep, and creeps
On shelving shores, or climbs on rocky steeps.
As in dry air the sea-born stranger roves,
Each muscle quickens, and each sense improves;
Cold gills aquatic form respiring lungs,
And sounds aerial flow from slimy tongues⁵³.

The pressures that shape evolution are expressed with equal verve as he describes the struggle for existence among animals, insects, fish and, here, plants:

Yes! smiling Flora drives her arm'd car
Through the thick ranks of vegetable war;
Herb, shrub, and tree, with strong emotions rise
For light and air, and battle in the skies;
Whose roots diverging with opposing toil
Contend below for moisture and for soil⁵⁴.

"Where shall we find a benevolent idea to console us" amid so much slaughter, he asks. The answer is provided by his idea of organic happiness: he reminds us that

when a Monarch or a mushroom dies,
Awhile extinct the organic matter lies;
but it soon teems with microscopic life again—
Reproduction strives
With vanquish'd Death—and Happiness survives.

Invention and research

Concentrating on Darwin's books, as I have done, brings out the variety of his accomplishments, but leaves unrecorded his contributions to technology and science. His Lunar Society friends looked on Darwin as primarily an inventor, and Boulton made a contract to pay him £1,000 for a developed version of his speaking-machine, which could pronounce many words "with so great nicety as to deceive all who heard it unseen". He constructed a mechanical "bigrapher", an early office copying machine, which worked well and inspired Watt to invent a chemical copying method "which beats your bigrapher hollow". He designed a number of water machines, including some elaborate pumps, an automated water closet, a wire-drawn ferry and a canal lift (Fig. 2), identical in design with those brought into operation 100 years later. Other paper designs include an artificial bird with flapping wings powered by a compressed-air bottle (Fig. 3), a sketch of a hydrogen-oxygen rocket motor, and telescopes made of numerous small lenses or mirrors: all three of these have proved prophetic⁵⁵.

Darwin did not devote much time, except in his medical work, to what we would call scientific research, but he still managed to achieve more than most scientists living today can hope for. His first two papers in the *Philosophical Transactions* are of little interest now, but they secured him election as a Fellow of the Royal Society in 1761, the first of five generations of Darwins to become Fellows. In one of his later papers, in 1785, he offers the first proper explanation of the principle of the artesian well⁵⁶. But it is in his last paper in the *Philosophical Transactions*, in 1788, that he makes his

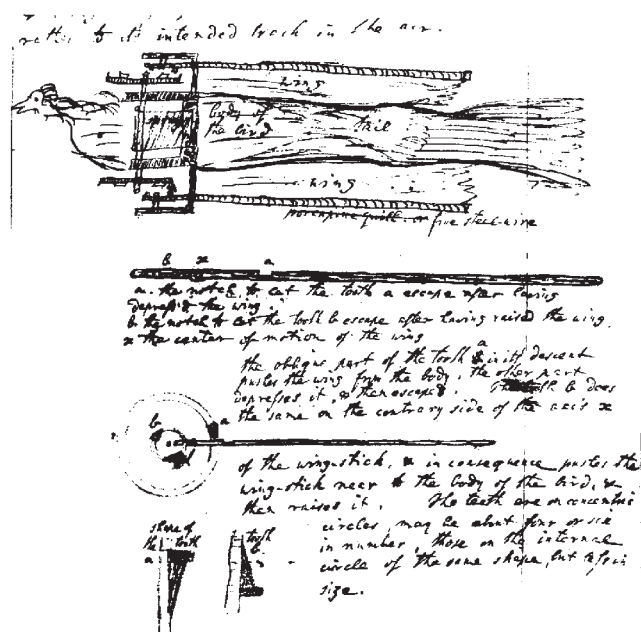


Fig. 3 Darwin's artificial bird (1777). In this version the model is energised by a wound spring, but Darwin suggests a compressed-air bottle as an alternative source of power. Reproduced by courtesy of The Royal College of Surgeons of England.

most important contributions to physical science. In this paper⁵⁷ he gives the first clear statement of the principle of adiabatic expansion⁵⁸, fundamental to all heat engines: his discovery sprang from long familiarity with steam engines and Watt's problems in improving them. Darwin then applied the principle to explain the formation of clouds: "when large districts of air are raised . . . they become so much expanded by the great diminution of pressure over them . . . that . . . the air by its expansion produces cold and devaporates"⁵⁷. Meteorology was a lifelong interest with him, and in addition to this discovery of the main mode of cloud formation he also recognised the existence of cold and warm fronts and of "rolling cylinders of air" in the lee of mountains¹⁰.

In this article I have mentioned thirty-six subjects to which Darwin made pioneering contributions. I have left unmentioned thirty-nine others, including abolition of slavery, aesthetics, afforestation, animal camouflage, artificial insemination and aurorae—to quote only those beginning with 'a'. Of Darwin we can truly say, "We shall not look upon his like again". Yet schoolchildren and undergraduates in English-speaking countries complete their 'education' without hearing anything of Erasmus Darwin—a paradox for pedagogues and publishers to ponder. And, now that Darwin's poems are in print again, librarians have it in their power to popularise him.

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Noradrenaline binding and the search for catecholamine receptors

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The binding of ^3H -noradrenaline to intact cells and microsomes seems not to measure catecholamine receptor interactions, but rather a membrane catechol-binding protein which may be related to the enzyme, catechol-O-methyl transferase. True receptors are probably scarce and detection will require catecholamine derivatives of much higher specific activity than are at present available.

In the past few years considerable progress has been made in the identification of membrane-bound receptor structures for polypeptide hormones (insulin, glucagon, ACTH) in mammalian tissues and for cholinergic drugs in specialised tissues of certain fish. These studies have been based on the selective binding of highly radioactive (about $1 \text{ Ci } \mu\text{mol}^{-1}$) hormone derivatives to isolated cells or membrane preparations. These interactions demonstrate very strict specificity, saturability indicative of a finite and limited number of receptors, reversibility, and affinity and kinetic rate constants of binding which correlate with the biological properties of the hormone. Although radioactively labelled catecholamines have been used successfully for many years in the elucidation of important transport, storage and binding functions of adrenergic neurones¹⁻¹⁰, only recently have these labelled compounds been used to measure putative β -adrenergic receptors. Interactions between ^3H -labelled catecholamines and receptors have been described for isolated liver¹¹⁻¹³ and heart¹⁴⁻¹⁷ microsomal preparations, spleen capsule¹⁸, cultured myocardial cells¹⁹, and turkey erythrocyte ghosts^{20,21}. Here we describe our attempts to identify β -adrenergic receptors in intact cells and in microsomes from various tissues. Our data indicate that the binding of ^3H -noradrenaline does not measure catecholamine receptor interactions but rather a membrane catechol-binding protein which may be related to the enzyme, catechol-O-methyl transferase (COMT).

The binding of ^3H -(-)-noradrenaline to microsomal preparations (see legend to Table 1) from liver, heart and fat cells is a saturable process with respect to the hormone, although the binding is complicated by the existence of at least two separate binding processes and by an apparent cooperativity at low hormone concentrations. In microsomes stored at -20°C for 1 month the first plateau of binding indicates a capacity of about 1.4 nmol of hormone per mg of membrane protein in liver, 1.1 nmol in fat cell and 1.7 nmol in heart microsomes. These binding capacities are 10^3 to 10^4 times larger than for insulin^{23,25} and glucagon^{27,28} binding to fat and liver cell membranes. Using intact isolated fat cells, which are especially useful because of the homogenous nature

of the cell type, absence of neural tissue and exquisite sensitivity to catecholamines, the data indicate a maximal ^3H -(-)-noradrenaline binding capacity of about 5×10^6 molecules per cell, which is at least 500 times greater than the binding capacity of these cells for insulin²². Myocardial cells in culture bind about 2.8×10^6 molecules per cell¹⁹. The concentration of unlabelled noradrenaline required to decrease the binding by 50% in fat, liver and heart cell microsomes and in intact fat cells is about 10^{-6} M .

These data must be interpreted very cautiously because the binding capacity of microsomes (in liver, heart and fat cells) increases strikingly during storage. Storage of fresh liver microsomes at -20°C in 50 mM sodium phosphate buffer resulted in a seven-fold increase in binding after 8 d and a fifteen-fold increase after 17 d; little further change occurs after 30 d. Storage of fresh liver microsomes at 4°C in 0.1 M Tris-HCl, pH 8.6, for 16 h results in a nine-fold increase in binding. Binding to stored fat cell microsomes, relative to insulin binding, is at least 200 times greater in the microsomes than in the intact cells.

Non-radioactive (+)- and (-)-noradrenaline compete in an identical way with ^3H -(-)-noradrenaline for binding to intact fat cells. The apparent affinity constants estimated from such inhibition studies are about 1 to $3 \times 10^{-6} \text{ M}$. The apparent affinity constant calculated from dose-response studies of lipolysis (see legend to Table 1) is about $2 \times 10^{-7} \text{ M}$. If sodium metabisulphite ($20 \mu\text{g ml}^{-1}$) is added to the medium to prevent oxidation of the catecholamine during the incubation (60 min, 37°C), the lipolytic dose response curves become hyperbolic and a half-maximal response was obtained at $6.5 \times 10^{-8} \text{ M}$. Thus, there are substantial differences (about thirty-fold) between the apparent affinities for binding and for activation of a biological response in the identical, intact cell system.

The complete lack of stereospecificity for binding of ^3H -(-)-noradrenaline has been repeatedly confirmed in numerous systems including microsomal preparations from rat liver, heart, spleen and fat cells, sheep spleen capsule and pineal gland, and rabbit superior cervical ganglion. Even small degrees of stereospecificity are not detectable despite numerous variations in the concentration of ^3H -(-)-noradrenaline, temperature and time of incubation, rapidity of preparation of tissue, and composition of the buffer and cations. Others have also reported that the binding of catecholamines to liver¹³ and heart¹⁷ microsomes and turkey erythrocyte ghosts²¹ is not stereospecific.

Specificity for catechols

Without exception, all compounds having a 3,4-dihydroxy-phenolic moiety can effectively inhibit the binding of ^3H -(-)-

noradrenaline to microsomal preparations (Table 1). No reproducible differences were detectable in the relative ability of a widely divergent group of catechols to compete for binding. Pyrocatechol and dihydroxymandelic acid, which are not catecholamines, can displace binding effectively and completely. The presence of a third hydroxyl group at position 6 of the catechol ring (6-hydroxydopamine) resulted in an eight-fold lower apparent affinity. Compounds related to biogenic amines but lacking a catechol group are ineffective. Very similar results were obtained with microsomes from fat cells, liver and heart. The specificity of binding is thus restricted to catechol derivatives. This requirement for the catechol structure has also been reported for liver^{12,13} and heart¹⁷ microsomes and for turkey erythrocyte ghosts²¹.

(+)-Noradrenaline, which can completely block the binding of ³H-(−)-noradrenaline as effectively as (−)-noradrenaline, was ineffective in eliciting lipolytic responses in fat cells at concentrations (5 µg ml^{−1}) more than 1,000 times greater than

Table 1 Competition by Various Compounds with Binding of ³H-(−)-Noradrenaline to Liver Cell Membranes

Addition	Concentration (µg ml ^{−1})	Specific c.p.m. bound
None		17,700 ± 500
(−)-Noradrenaline	0.5	9,200 ± 200
	1.5	4,900 ± 100
(+)-Noradrenaline	0.5	9,000 ± 300
(−)-Isoproterenol	0.5	8,600 ± 400
	1.5	4,800 ± 300
Dopamine	0.5	6,900 ± 500
	1.5	4,100 ± 200
α-Methylnoradrenaline	0.5	8,800 ± 300
3,4-Dihydroxymandelic acid	0.5	7,500 ± 400
	1.5	4,600 ± 300
Pyrocatechol	0.5	8,500 ± 200
	1.5	4,900 ± 300
6-Hydroxydopamine	1.5	14,100 ± 200
	5.0	8,600 ± 300
	10.0	5,100 ± 100
(−)-Phenylephrine	50	16,800 ± 400
Tyramine	50	17,500 ± 500
Propranolol	500	14,200 ± 500
Metanephrine	50	17,200 ± 300
Phentolamine	50	16,900 ± 500
Phenylethylamine	50	17,400 ± 400

Triplicate samples (0.25 ml) containing 0.3 mg of protein per ml of Krebs-Ringer-bicarbonate (KRB) buffer (pH 7.4) were preincubated (24° C) for 3 min with the indicated compound, 0.2 µM ³H-(−)-noradrenaline (2.6 × 10⁵ c.p.m., 6.4 Ci mmol^{−1}) was added and incubations were continued for 30 min at 24° C. Binding was determined by Millipore membrane (cellulose acetate) filtration, which allows the samples to be cooled (4° C), filtered and washed (4° C) within 10 s, thus decreasing the probability of dissociation of the complex^{22,23}. For all samples controls were performed in which unlabelled (−)-noradrenaline (50 µg ml^{−1}) was added before ³H-(−)-noradrenaline; this binding was considered non-specific, and was subtracted to calculate specific binding^{22,23}. Non-specific binding is generally less than 0.5% of the radioactivity in the medium and less than 10% of the total bound except in fat cells and turkey erythrocytes and ghosts where at high (10^{−6} M) hormone concentrations a larger part may be non-specific. Liver membranes (Sprague-Dawley rats) are the microsomal pellet of homogenates prepared in 0.25 M sucrose^{24,25}. Fat cell membranes represent the total particulate fraction of intact fat cells homogenised in Krebs-Ringer-bicarbonate buffer²⁶. Rat heart microsomes were prepared from homogenates (Polytron, 3 min, 0.25 M sucrose) by the centrifugation procedures described^{14,17}, which result in very low yields of material, as well as by collecting the 2,500g (15 min) pellet after discarding the material, sedimenting at 300g (15 min); the results were similar with both preparations. Intact turkey erythrocytes were used after washing thoroughly with ice-cold 50 mM sodium phosphate, pH 7.4, and suspending in KRB buffer, pH 7.0. Ghosts were prepared each day by freezing and thawing followed by washing as described above; the ghosts are devoid of haemoglobin and most are nucleated. Glass fibre paper filters (Whatman, GF/C 2.4 cm) were used in the filtration assay of erythrocytes and ghosts since the high concentrations (1 to 8 × 10⁸ cells ml^{−1}) required to detect binding caused plugging of Millipore filters. All binding studies were performed in KRB buffer (pH 7.4), although generally similar findings were obtained with phosphate buffers.

those of (−)-noradrenaline which cause detectable lipolysis (Table 2). Furthermore, the (+)-isomer did not modify at all the lipolytic response to (−)-noradrenaline, even when its concentration was 1,000 times greater. Other catechols which effectively compete for binding, which possess virtually no intrinsic β-adrenergic activity in fat cells, and which do not modify the activity of (−)-noradrenaline or (−)-isoproterenol include dopamine, pyrocatechol (Table 2) and 3,4-dihydroxymandelic acid.

Table 2 Lipolytic Activity of Catechol Substances, and their Effect on the Lipolytic Activity of (−)-Noradrenaline in Isolated Fat Cells

Addition	Concentration (ng ml ^{−1})	Glycerol released
None		13.6
(−)-Noradrenaline	10	48.7
	50	71.0
(−)-Isoproterenol	0.5	27.4
(+)-Noradrenaline	500	14.4
	5,000	14.6
Pyrocatechol	2,000	14.5
Dopamine	500	16.2
3,4-Dihydroxymandelic acid	500	15.1
(+)-Noradrenaline + (−)-Noradrenaline	10	51.4
(−)-Isoproterenol	0.5	28.2
Pyrocatechol + (−)-Noradrenaline	2,000	49.8
Dopamine + (−)-Noradrenaline	500	52.9
3,4-Dihydroxymandelic acid + (−)-Noradrenaline	500	50.1
	10	

Fat cells were incubated for 80 min in KRB buffer-3% albumin, 7 µg per ml of sodium metabisulphite, and the indicated compound. Lipolysis was determined by measuring the release of glycerol in the medium^{30,31}. Results are expressed as µmol of glycerol per mmol of cell triglyceride. The lipolytic potency of (−)-phenylephrine, pronetholol, phentolamine, and 6-hydroxydopamine was at least 100 times lower than that of (−)-noradrenaline while the potency of (−)-isoproterenol was five to ten times greater.

(+)-Noradrenaline did not stimulate the activity of adenylate cyclase in microsomes of isolated fat cells except at concentrations 200 to 1,000 times (depending on the batch) greater than those of (−)-noradrenaline (Fig. 1). This probably resulted from trace contamination with the (−)-isomer. The (+)-isomer in ten- to fifty-fold molar excess did not inhibit the activity stimulated by (−)-noradrenaline (Fig. 1). The catechol compounds, dihydroxymandelic acid, α-methyl dopa and pyrocatechol did not stimulate adenylate cyclase activity at concentrations as high as 10^{−4} M, and they did not inhibit (at 10^{−4} M) the stimulation (5.5-fold) caused by 5 × 10^{−6} M (−)-noradrenaline.

In liver microsomes maximal stimulation of adenylate cyclase (conditions as in Fig. 1) occurred with 10^{−5} M (−)-noradrenaline. Concentrations higher than 0.1 mM reversed the stimulation, presumably non-specifically. (+)-Noradrenaline did not inhibit the (−)-isomer in liver, even at 100-fold higher concentrations, provided its concentration did not exceed 1 mM. Such high (+)-noradrenaline concentrations were non-specifically toxic since they also inhibited activities stimulated by glucagon and fluoride. Fluoride (10 mM) stimulation was inhibited about 18% and 60%, and glucagon (10^{−6} M) was inhibited 20% and 100%, by 1 mM and 10 mM, respectively, of (+)-noradrenaline.

The non-catechol, *m*-methanesulphonamide derivative of isoproterenol, Soterol (compound number 49)^{32,33}, stimulated lipolysis in fat cells and adenylate cyclase activity in fat cell membranes, and its activity was blocked by propranolol. Its apparent affinity was similar to that of (−)-noradrenaline. Despite this, very high concentrations (0.5 mM) of Soterol (gift from Dr W. T. Comer, Mead Johnson) only decreased

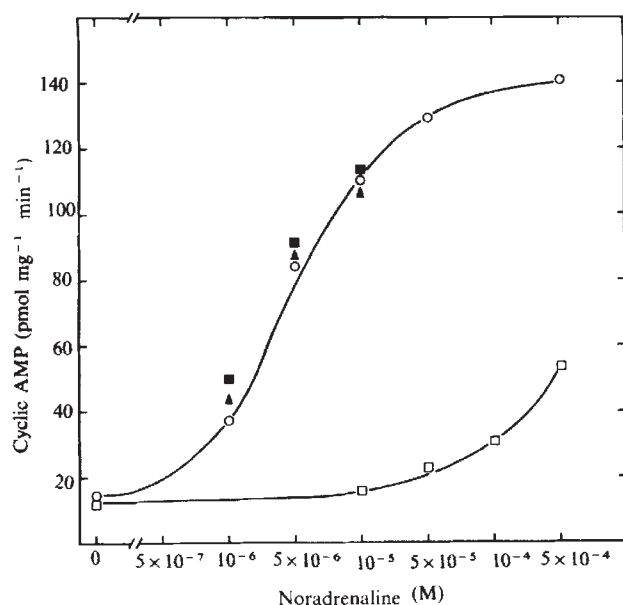


Fig. 1 Effect of (+)-noradrenaline on (-)-noradrenaline stimulation of adenylate cyclase activity in isolated fat cell microsomes. The incubation mixture (0.1 ml) contained 25 mM Tris-HCl (pH 7.6), 5 mM MgCl₂, 0.1 mM MnCl₂, 0.1 mM GTP, 2.5 mM aminophylline, 0.1% (w/v) albumin, 0.1 mM α -³²P-ATP (6×10^6 c.p.m.) and 100–200 μ g of membrane protein; 5 mM phosphoenolpyruvate and 60 μ g ml⁻¹ of pyruvate kinase were used to regenerate ATP. After 5 min at 37° C, the tubes were placed in boiling water for 2 min. A recovery mixture (1.0 ml) containing ³H-cyclic AMP was added to each sample. Cyclic AMP was isolated on a column containing 1 g of alumina that was eluted with 2.0 ml of 25 mM Tris-HCl (pH 7.6). Values are averages of triplicate replications. □, (+)-Noradrenaline; ○, (-)-noradrenaline; ▲, (-)-noradrenaline with 10^{-5} M (+)-noradrenaline; ■, (-)-noradrenaline with 5×10^{-5} M (+)-noradrenaline.

the binding of ³H-(+)-noradrenaline (0.2 μ M) by only 13% in fat cells, 14% in liver, and 22% in heart microsomes.

By two different methods the ³H-(+)-noradrenaline which binds to liver, heart and fat cell membranes could not dissociate over a 40- to 80-min period at 4° C, 24° C, or 37° C (Fig. 2). The results were similar if the hormone-membrane complex was incubated for only 10 min at 24° C or 4° C before dissociation was studied, and this lack of dissociation was observed with fresh or stored microsomes. These results contrast with the immediacy of reversal of the catecholamine effect observed upon removal of the hormone from the medium in a variety of biological systems, including fat cells. HCl (0.4 N) did not reverse but instead slightly enhanced the complex between noradrenaline and liver or heart microsomes (Fig. 2, insert). Guanidine-HCl (5 M) increased, and 0.4 N NaOH depressed, the binding. The essentially irreversible nature of the binding and the facile oxidation of catechols have hindered chemical identification of the membrane-bound ³H.

Relation with enzyme COMT

In agreement with others^{13,17}, proteolytic (Pronase) digestion of membranes destroyed subsequent binding activity, indicating that protein components are involved. The specificity of binding described above is very different from that observed for the transport, storage and binding functions of adrenergic neurones or storage vesicles^{1–10}; furthermore, isolated fat cells and erythrocytes are devoid of such structures.

The strict requirement of the binding process on the presence of a catechol function suggests a possible relationship with the enzyme, catechol-O-methyl transferase (COMT). This enzyme has similar affinities for a wide variety of catechols,

even for those lacking a ring side chain, and it does not distinguish between (+)- and (-)-forms of noradrenaline^{34–37}. Although COMT is present in the soluble fraction of most tissues, its presence in microsomes is well established^{38–40}. In isolated fat cells the enzyme is found predominantly in a membrane-bound form⁴⁰. Although the particulate and soluble enzymes differ in certain respects³⁸, both demonstrate broad substrate specificity^{38,39}.

The presence of COMT activity^{34,35} in the liver, fat and heart microsomal preparations used in these studies was confirmed using ³H-(+)-noradrenaline or ¹⁴C-S-adenosyl-methionine⁴¹ as substrates. As observed for COMT^{13,17}, reagents that blocked sulphhydryl groups (such as *p*-chloromercuribenzoate) also blocked ³H-(+)-noradrenaline binding. Also like COMT, (+)-noradrenaline binding required divalent cations; binding decreased 50% with 2 mM EDTA in liver membranes. Some well known^{37,42,43} inhibitors (tropolone, pyrogallol, quercetin and U-0521) of COMT which do not have β -adrenergic activity could also inhibit the binding to liver and heart microsomes (Table 3).

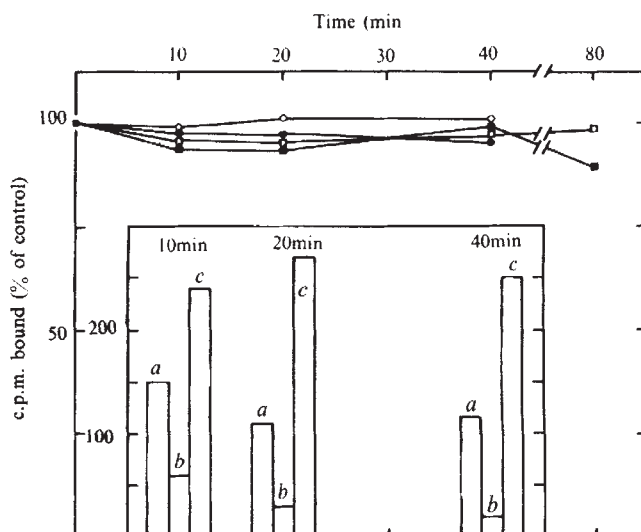


Fig. 2 Absence of spontaneous dissociation of the complex formed between ³H-(+)-noradrenaline and heart (○, □) and liver (●, ■) microsomes, and the effect of HCl, NaOH and guanidine-HCl on dissociation of the complex. Microsomes were incubated (see Table 1) at 24° C for 60 min with 1.5×10^6 c.p.m. ml⁻¹ (0.2 μ M) of ³H-(+)-noradrenaline (6.4 Ci mmol⁻¹). At this time unlabelled (+)-noradrenaline (50 μ g ml⁻¹, 0.3 mM) was added and the samples were incubated at 37° C (●, ■) or 24° C (○, □) to measure the rate of dissociation²⁶. The effect of adding, a, 0.4 N HCl, b, 0.4 N NaOH and, c, 5 M guanidine-HCl after the 60 min period of incubation is shown in the insert. Spontaneous dissociation was also negligible when measured by thoroughly removing the excess hormone in the medium by washing on Millipore filters and replacing with fresh medium²². Similar results are obtained if the period of preincubation is short (10 min), or if the temperature of preincubation is 4° C.

S-Adenosyl-L-methionine (SAM), a compulsory substrate for COMT activity^{34,35,43}, markedly increased (two- to four-fold at 1 mM) the rate of binding of ³H-(+)-noradrenaline to liver and heart microsomes (Fig. 3); effects were detectable at concentrations below 0.1 mM. The effect of SAM was most pronounced on freshly prepared microsomes, and little effect could be observed in preparations stored frozen for more than 3 weeks. SAM did not dissociate bound radioactivity even if added after removal of the free ³H-(+)-noradrenaline in the medium. Thus, SAM did not simply increase the rate of turnover of the catecholamine-membrane complex. The normal product of SAM hydrolysis by COMT, S-adenosyl-homocysteine (SAH), inhibited weakly the binding to heart and liver microsomes; 10% to 20% inhibition (measured at 10 min, 24° C) occurred in the presence or absence of SAM (1 mM) with 5 mM SAH. The normal (+)-noradrenaline

Table 3 Effect of Inhibitors of Catechol-O-Methyl Transferase on Binding of ^3H -(-)-Noradrenaline to Liver Membranes

Addition	Concentration ($\mu\text{g ml}^{-1}$)	Specific ^3H -noradrenaline bound (c.p.m.)
None		8,200 $\pm$ 300
(-)-Noradrenaline	2.5	3,300 $\pm$ 100
	0.5	5,400 $\pm$ 300
	0.1	7,600 $\pm$ 200
Tropolone	0.5	5,900 $\pm$ 100
	0.1	6,800 $\pm$ 200
U-0521	10	6,300 $\pm$ 100
	0.5	7,300 $\pm$ 300
Pyrogallol	10	3,600 $\pm$ 100
	0.5	6,100 $\pm$ 200
Quercetin	10	3,100 $\pm$ 200
	0.5	6,100 $\pm$ 300

Membranes (0.2 ml containing 1.1 mg of protein per ml) were incubated (24°C) for 3 min with the indicated compound followed by 60 min at 4°C with $0.9 \mu\text{M}$ ^3H -(-)-noradrenaline (4.8×10^5 c.p.m., 6.4 Ci mmol^{-1}) as described in Table 1. Similar results were obtained by incubating for 30 min at 24°C , and with heart microsomes. U-0521 is 3,4-dimethoxy-5-hydroxybenzoic acid³⁷.

product of transferase activity, (-)-normetanephrine, did not alter the binding (including rates) of ^3H -(-)-noradrenaline ($0.1 \mu\text{M}$) in the absence or presence of SAM even when tested at concentrations as high as 0.3 mM . The material bound to membranes was probably not a simple product of COMT catalysis.

The close analogue of SAM, S-adenosylethionine (SAE), profoundly decreased the binding of ^3H -(-)-noradrenaline to heart and liver microsomes (Table 4). Significant effects occurred with $5 \mu\text{M}$ SAE. SAE could decrease the binding even more effectively than nearly saturating concentrations ($5 \mu\text{g ml}^{-1}$) of unlabelled (-)-noradrenaline. Inhibition was observed only if SAE is added before ^3H -(-)-noradrenaline, and no dissociation was seen if it was added after the complex has incubated for 10 to 30 min at 24°C or 10 to 60 min at 4°C , even if the microsomes were washed before testing. SAE abolished the effects of SAM described above, and Dixon plots suggested competitive kinetics of inhibition with an apparent dissociation constant of about $80 \mu\text{M}$ (heart microsomes). SAE is a potent inhibitor of liver and heart microsomal COMT activity. When assayed with $30 \mu\text{M}$ ^{14}C SAM and 1 mM 3,4-dihydroxybenzoic acid (under conditions giving zero order kinetics), concentrations of SAE of 0.3 mM , $30 \mu\text{M}$, and $3 \mu\text{M}$ inhibited COMT activity of liver microsomes by 85%, 56% and 15%, respectively.

Table 4 Inhibition of Binding of ^3H -(-)-Noradrenaline to Heart and Liver Membranes by S-Adenosylethionine

Addition	Total ^3H -noradrenaline bound (c.p.m.)	
	Liver microsomes	Heart microsomes
None	13,500	7,200
(-)-Noradrenaline, $5 \mu\text{g/ml}$	800	600
S-Adenosylethionine, 5 mM	500	400
	1 mM	850
	0.2 mM	1,250
	$40 \mu\text{M}$	5,900
	$5 \mu\text{M}$	9,300

The experiments were performed as described in Fig. 3. Incubations (24°C , 40 min) (0.2 ml) contained 0.24 mg (liver) or 0.30 mg (heart) microsomal protein and 1.2×10^5 c.p.m. (liver) or 2.0×10^5 c.p.m. (heart) of ^3H -(-)-noradrenaline (6.4 Ci/mmole).

3-Mercaptotyramine, which selectively and irreversibly inactivates soluble COMT presumably by forming disulphide complexes at the active site of the enzyme⁴⁵, is a very potent

inhibitor of noradrenaline binding to liver and heart microsomes. Incubation of heart microsomes for 15 min (37°C) with 0.3 mM , $30 \mu\text{M}$, $6 \mu\text{M}$, $1 \mu\text{M}$ and $0.3 \mu\text{M}$ 3-mercaptopotyramine (a gift from Dr C. R. Creveling) decreased the subsequent binding (30 min, 24°C) of ^3H -noradrenaline ($0.8 \mu\text{M}$) by 100%, 91%, 73%, 28%, and 15%, respectively.

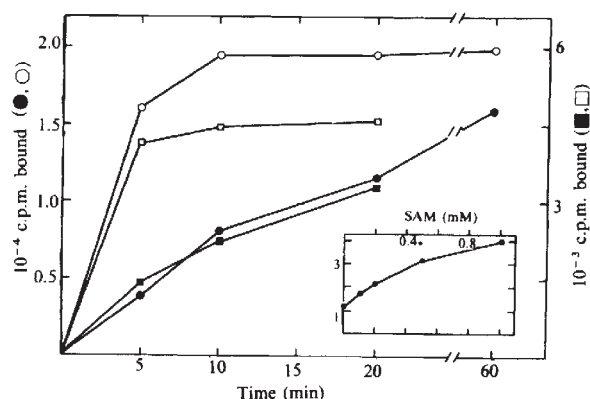


Fig. 3 Effect of S-adenosylmethionine (SAM) (○, □) on the binding of ^3H -(-)-noradrenaline (4.5×10^5 c.p.m. for heart, 1.9×10^5 c.p.m. for liver; 6.4 Ci mmol^{-1}) to liver (●, ○) and heart (■, □) microsomes. SAM was added immediately before the radioactivity. The protein content was 0.24 (heart) or 0.21 (liver) mg per 0.2 ml , and incubations were at 24°C . Insert shows the effect of SAM concentration on heart microsomes measured at 5 min.

Since noradrenaline binding is essentially irreversible, and since SAM does not release the bound material, the binding may represent an aberrant, covalent enzyme-substrate intermediate which is not susceptible to completion by the normal catalytic process. The very large increase in binding capacity during storage of microsomes or by treatment with Tris-HCl may reflect such an alteration of the enzyme. It may be relevant that dithiothreitol (DTT) potentially inhibits the binding to liver and heart microsomes. Binding (30 min, 24°C) of $0.4 \mu\text{M}$ ^3H -(-)-noradrenaline to heart microsomes is decreased by 82%, 73%, 48% and 15% by 1 mM , 0.25 mM , $60 \mu\text{M}$ and $15 \mu\text{M}$, respectively, of DTT. Although the activity of soluble COMT (heart or liver) is not inhibited by 3 mM DTT, the activity of microsomal, particulate preparations is enhanced four- to six-fold by concentrations of DTT as low as 0.6 mM . DTT thus may revert an altered particulate enzyme into a form that no longer binds catechol substrates irreversibly.

Solubilisation of catechol binding protein

As described by others^{13,15,16}, the binding protein can be solubilised from microsomes with non-ionic detergents. We studied extensively the material solubilised from fat and liver microsomes with Triton X-100. The soluble protein- ^3H -noradrenaline complex would be assayed by precipitation with 65% $(\text{NH}_4)_2\text{SO}_4$, followed by Millipore filtration as described for microsomes, or by gel filtration on Sephadex G-25. As in microsomes, binding was destroyed by Pronase, was saturable with two distinct plateaus (in liver, K_d values were about $3 \times 10^{-7} \text{ M}$ and $2 \times 10^{-5} \text{ M}$), was suppressed by unlabelled hormone, was lacking in stereospecificity, demonstrated specificity only for catechols, and could not be dissociated even during 2 h at 37°C . Binding was inhibited by SAE ($50 \mu\text{M}$ inhibited the binding of $0.3 \mu\text{M}$ ^3H -noradrenaline to liver extracts by 75%), and the rate of binding was increased by SAM. 3-Mercaptotyramine⁴⁵ inhibited binding profoundly; 6 and $1 \mu\text{M}$ (15 min , 37°C) inhibited subsequent binding (liver) by 97% and 84%, respectively. Binding was also very sensitive to DTT. A most unusual property was the ability

of 5 M guanidine-HCl and 2% sodium dodecyl sulphate to increase binding by 50% and by two- to five-fold, respectively; unlabelled hormone (10 µg per ml) prevented this increased binding, which required tissue extract, and ^3H -noradrenaline did not bind to the detergent or to guanidine. Binding was suppressed in 2 M NaCl and in 8.3 M guanidine-HCl, pH 1. The complex, once formed, did not dissociate with 2% sodium dodecyl sulphate, 0.5 N HCl, or by boiling for 4 min.

Binding to turkey erythrocytes

In accordance with the report of Bilezikian *et al.*²¹, binding of ^3H -(-)-noradrenaline to turkey erythrocyte ghosts (see legend, Table 1) was not stereospecific and only required the catechol moiety. As in microsomes, binding was only very weakly inhibited by Soteranol, and it was inhibited by SAE and by 3-mercaptotyramine (although higher concentrations were required). Low concentrations (0.2 mM) of DTT inhibited binding, but not as profoundly as in microsomes. Unlike liver and heart microsomes, the bound ^3H -noradrenaline could dissociate spontaneously.

There were striking differences between the binding to intact erythrocytes and to ghosts. Freshly prepared ghosts bound thirty to seventy times more noradrenaline (per cell) than intact cells. Storage of the ghosts (-20°C , 3 d) further increased binding by a factor of two to three; binding did not change upon storing intact cells at 4°C for 3 d. An intact cell could bind a maximum of about 14,000 noradrenaline molecules, while a ghost could bind about 650,000. Intact human erythrocytes, which are unresponsive to catecholamines⁴⁶, bound about 5,000 molecules per cell.

Maximal stimulation (seven-fold) of adenylate cyclase activity (methods as in Fig. 1) in ghosts occurred with $5 \times 10^{-5}\text{ M}$ (-)-noradrenaline; concentrations higher than 0.5 mM reversed the stimulation, as described for microsomes. The activity of the (-)-isomer was not reversed by 100- to 500-fold higher concentrations of (+)-noradrenaline provided these did not exceed 10^{-3} M , which, as described earlier, non-specifically suppress activity. Pyrocatechol, dihydroxymandelic acid and α -methyl dopa did not stimulate the enzyme, and these compounds at 10^{-4} M and $5 \times 10^{-4}\text{ M}$ did not inhibit the stimulation (5-fold) of $5 \times 10^{-6}\text{ M}$ (-)-noradrenaline. The isoproterenol (10^{-8} M) enhanced rate of sodium outflux (15 min) in turkey erythrocytes was not modified by a 1,000-fold molar excess of DL-dopa, dopamine, vanilmandelic acid, or 3,4-dihydroxyphenylacetic acid, compounds that completely block the binding of ^3H -isoproterenol⁴⁶. Although these compounds (1,000-fold excess, 0.2 mM) decreased the levels of cyclic AMP stimulated (90 min) by isoproterenol, the inhibition was only about 15%, and possible non-specific toxicity was not examined⁴⁶.

Stereospecific binding was not demonstrable in intact erythrocytes, despite the low number of binding sites compared to ghosts. The residual binding observed in the presence of pyrocatechol, 3-mercaptotyramine, SAE or DTT did not reveal stereospecificity. Despite differences in the binding between turkey erythrocytes (and ghosts) and mammalian cells or microsomes, the essential features indicate that the binding did not reflect a true β -adrenergic receptor interaction. The possible relation of binding to catechol transport has not been examined.

The binding of ^3H -(-)-noradrenaline to subcellular particles or cells cannot be reconciled with the view that such binding reflects β -adrenergic receptor interactions. The single most compelling argument is that certain compounds which completely prevent this binding have no intrinsic biological activity and do not measurably alter the biological activity of (-)-noradrenaline. Binding is probably related to a totally separate process which shares with catecholamine receptors only the property of recognising catechol moieties. It must be concluded that if such putative receptors exist in the materials

examined, they must represent a negligible fraction of the actual binding of ^3H -noradrenaline observed. Estimates of the possible number of receptors in fat cells from lipolytic studies using a catecholamine, N-[2-(*p*-hydroxyphenylpropyl)]-(-)-noradrenaline (gift from Dr J. van Dijk, Philips-Duphar, The Netherlands), of very high apparent affinity (about $3 \times 10^{-10}\text{ M}$), with the assumption that 10% of the molecules in the medium are bound, give a maximum figure of 10^5 sites per cell. This is five to ten times greater than the maximum number of insulin or glucagon molecules that are bound per fat cell²². The numbers of noradrenaline binding sites in microsomes or cells are orders of magnitude greater than the corresponding figures for peptide hormones. It may therefore not be surprising that specific receptors cannot be detected in the presence of other binding processes which exist in great excess. In turkey erythrocytes, however, the number of binding sites is very low. Assuming a cell radius of 4 µm, intact turkey cells have a binding density of about seven sites per µm², and ghosts about 300 sites per µm². (The density of insulin receptors in fat cells is 1 to 5 per µm², depending on cell size²².) But despite the scarcity of interfering catechol-binding sites in erythrocytes, stereospecific binding cannot be detected.

Many attempts to detect stereospecific binding in cells and membranes after experimentally suppressing (with pyrocatechol, specific inhibitors of COMT, SAE, 3-mercaptotyramine, DTT, EDTA and treatment with *p*-chloromercuribenzoate or iodoacetamine) the catechol-binding structures have failed. In these cases the residual binding is very low. This is consistent with the probable scarcity of true receptors. Detection will probably require catecholamine derivatives of much higher specific activity than are at present available. The ^{125}I -labelled peptide hormones used to study receptors are 100 to 300 times more radioactive than ^3H -(-)-noradrenaline.

For technical reasons the ligand used to detect receptors should have the highest possible affinity. It can be calculated from $K_d = k_{-1}/k_1$ (K_d , dissociation constant; k_1 and k_{-1} , rates of association and dissociation, respectively) that a complex having a k_1 of $5 \times 10^5\text{ mol}^{-1}\text{ s}^{-1}$, which is a very low estimate (ten times less than that of insulin receptors, 10^4 - to 10^5 -fold less than diffusion encounters), and a K_d of $5 \times 10^{-8}\text{ M}$ would exhibit a half-life of about 30 s. Since the true figure may well be ten times lower, it is possible that extensive dissociation of the complex can occur during washing of the particles or cells in the binding assay, especially if it is not performed at low temperatures.

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Typographical errors and technological solutions

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A recently reported typographical error in our global computer simulation model did exist, but had only a small quantitative effect on the published results. The error was not responsible for the pollution crisis mode; its removal does not stabilise the model system and the conclusions are unaffected by the numerical change.

In a recent issue of *Nature*¹, Boyle reports finding a typographical error in the computer simulation model used as a background for our book, *The Limits to Growth*². Boyle states that the error is responsible for the pollution crisis mode of the model and concludes that "affluence without restrictive social policies is apparently attainable". For the benefit of the many research groups currently working with our technical report on the model³, as well as the general public interested in the policy questions raised by *The Limits to Growth*, we would like to clarify the nature of this error and its effect on our conclusions.

Error

Tabular functions are often used in system dynamics models to express the nonlinear relationship of one variable

to another. In early versions of the world model we employed such a table function to represent the relative amount of pollution released as a function of the industrial output per capita. The table, called Pollution Generation Multiplier from Output Table (PGMOT), contained one mistyped value as indicated below (the values in the table express pollution relative to total global emissions in 1970, and they correspond to values of industrial output per capita over the range from \$0 to \$1,600 in steps of \$200).

$$T \text{ PGMOT} = 0/0.87/1.64/2.5/3.5/4.5/5.5/6.5/7.5$$

The mistyped number corresponds to a value of industrial output per capita of \$1,000 (equivalent to a GNP per capita of about \$4,000) and will cause an error in the model output whenever the value of industrial output per capita lies between \$800 and \$1,200. That situation occurs in three of the twelve model runs shown in *The Limits to Growth*, Figs 40, 41 and 42. In the other nine runs, industrial output per capita remains below the region where the PGMOT function is in error, and the error has no effect.

The typing error in PGMOT was corrected in our laboratory before the first draft of the technical report was completed in April 1972. To our knowledge, no research group working with any draft of the technical report has been given a program containing the error. We were under the impression that the figures in *The Limits to Growth* were drawn from the corrected version of the program, and we are indebted to Boyle for pointing out our mistake. The error, however, has no effect on the general message of the book and it does not alter our conclusions in any way.

That statement may be better understood if we examine the error in the full context of the model assumptions.

Implications

The basic assumptions of the world model are:

(1) Population and capital by their very nature tend to grow exponentially; the amount added to either population or capital each year is partially determined by the amount of population or capital already present. This exponential tendency is enhanced by current short-term, pro-growth value systems.

(2) There are limits to the growth of any physical quantity on a finite Earth. The carrying capacity for human population and industry is variable; it can be expanded by technology, and it can be diminished by misuse, erosion or depletion of resources.

(3) There are significant delays in the feedback processes that act to contain population and capital within the global carrying capacity. Some of these feedback delays arise from biological or physical processes (such as population ageing and the passage of a pollutant up a food chain) and others from social processes (such as perception and communication delays and resistance to change).

These three assumptions, considerably elaborated, constitute the world model. The conclusion that follows from them is that the present global population-capital system is dynamically unstable. The combination of rapid physical growth and long feedback delays can lead to a temporary overshoot of the sustainable physical limits to population and capital. During the overshoot period the carrying capacity can be eroded by overuse, leading to an eventual decline of population and industry. The overshoot can be entirely avoided by a change of values to favour a deliberate stabilisation of population and capital. The quality of life in the stabilised state can be greatly enhanced by technologies that preserve resources and abate pollution. Technological changes alone, however, unaccompanied by value changes that lead to deliberate slowing of growth, can lead to greater overshoot by temporarily enhancing exponential growth and by masking and further delaying the feedback pressures from physical limits.

This general conclusion is the most significant one to be drawn from the model or from *The Limits to Growth*. The model provides no information about whether the value changes that lead to stabilisation will actually occur. No single computer run of the twelve presented in *The Limits to Growth* can be interpreted as an exact prediction of the future state of the world—in fact the precise quantitative features of the model runs were deliberately removed to discourage such interpretation. The output of such a general, aggregate model can never be, and was never claimed to be, numerically accurate. Three different models, all built around the same assumptions but incorporating different degrees of detail and different numerical parameters, have produced quantitatively different but qualitatively identical conclusions²⁻⁴. These qualitative conclusions about system instability and the conditions that lead to stability are the only ones upon which any policy recommendations have been based.

Although the numerical error reported by Boyle does influence the quantitative output of three computer runs, it does not influence the system instability reflected in those runs, nor does it invalidate or even address any of the basic assumptions in the model. The version of Fig. 42, illustrated in Boyle's paper, still shows a severe overshoot of industrial capital, followed by an uncontrolled collapse. Boyle states, "The pollution crisis forecast . . . has been traced to a typographical error in the program" but pollution crises occur in Figs 36 and 37 of *The Limits to Growth*, where the error is not operative, and in several figures in both

World Dynamics (pages 10, 75, 96, 100, 105, 108–111) and the technical report³ (chapter VII, runs 7, 8, 13, 14) where the error is not even present.

Stabilisation policies

Boyle goes beyond the question of the typographical error to suggest an alternative set of policy changes that will stabilise the model system. Many such stabilising policy sets exist, each of which leads to a quantitatively different equilibrium state. The one common characteristic of these stabilising policies is that they supply some forward-looking social pressure against growth in place of the delayed-feedback physical pressures of the natural system.

The pressures that Boyle has selected—on agricultural development, capital investment, advertising and consumer demand for food and industrial output—are not different in kind from those we used to stabilise the model. (We called our stabilising measures "deliberate constraints on growth"; Boyle prefers to call his "rational economic policies".) The major difference is that he excludes any deliberate pressure on population growth. *The Limits to Growth* model considerably underestimates the growth potential of the human population, as discussed in detail in the technical report³ (chapter II) and so the question of whether policies that stabilise population in that model will stabilise a real population must be considered. In any event, *The Limits to Growth* was intended to raise the question of which pressures against growth will be effective and which we would prefer to live under, not to settle the question. Dr Boyle's alternative suggestion is a positive contribution to a debate that is just beginning.

We must take some issue, however, with Boyle's "real conclusion" that there is "hope for the technological solution". Even the crude representations of technology in *The Limits to Growth* model and in Boyle's paper do not lead to that conclusion. Furthermore, technology in this model is assumed to be immediate, perfectly effective, free of cost, and free of any unforeseen side effects. This lack of realism was utilised in *The Limits to Growth* only to illustrate the extreme case. In the technical report³ (chapter VII, runs 21, 22, 23) and in detailed working papers⁵ (chapters 3, 6 and 8), capital costs of technology and implementation delays are included. In these more realistic representations, reliance upon technology to bring an orderly transition to system equilibrium looks even less feasible.

Technological advance can, and we hope will, do much to make life more livable during the transition and in the stable state. We do not believe, however, that technology alone can produce a solution to problems arising from an inconsistent, short-term, anthropocentric set of values based primarily on the concept of an infinite Earth. Social problems ultimately require social solutions, not technological ones. It seems unpleasant for us to recognise the fact, but the sooner we do so, the sooner the necessary solutions are likely to be found.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Lepton Creation and the Dirac Relationship between Fundamental Constants

CAVALLO¹ has proposed an interpretation of the large number ratios between some of the fundamental constants occurring in physics and cosmology, in terms of rotating cosmological models. The purpose of this communication is to suggest another interpretation of these large dimensionless numbers in terms of lepton creation.

The starting point of this theory is the usual action principle for gravitation and electromagnetism but with the difference that charged leptons can be created or annihilated in the cosmological space-time. That is, the worldlines of these particles can have a beginning or an end, or both. The action can be written either in the field form or in the form of action at a distance². The variation of the worldline of a lepton of mass m and charge e gives the following condition at the beginning or end of a worldline:

$$m\mathbf{u}_i = -eA_i \quad (1)$$

where A_i is the four-potential from the rest of the particles in the Universe and $\mathbf{u}_i$ is the four-velocity of the lepton.

I now apply this to a Robertson-Walker space-time used by most cosmologists, and used by Dirac in his discussion³. This represents an expanding homogeneous and isotropic Universe. In any given cosmological model of this type, it is possible in principle to calculate the A_i from charges of one species, although in practice the relevant integral may not be expressible in terms of elementary functions⁴. But if the Universe as a whole is electrically neutral the total A_i appearing on the right hand side of equation (1) will be zero.

I now propose a departure from strict neutrality in the following way. Suppose we consider particles of electric charge e . Although cosmological models differ in detail (such as deceleration parameter, the value of the λ constant, big bang or steady state, and so on), only the time-like component of A_i from all these particles is non-zero and it has the magnitude of the order of

$$Ne/R \quad (R = c/H) \quad (2)$$

where N is the number of such charges in a spherical region of radius R . The parameters c and H in equation (2) are the velocity of light and the present value of the Hubble constant respectively. In the case of strict charge neutrality particles of opposite charge $-e$ will make an equal contribution $-Ne/R$ and thus cancel equation (2) exactly. But if the charge balance is a statistical effect, small fluctuations from it are likely to be present. This would mean that there is an overall electrostatic potential of the order

$$A_4 \sim \pm N^{1/2}e/R \quad (3)$$

The fluctuation effect assumed in equation (3) is of the order $N^{-1/2} (\sim 10^{-40})$.

Using equation (3) in equation (1), for a lepton created at rest in the cosmological substratum,

$$mc^2 = \mp N^{1/2}e^2/R \quad (4)$$

Since the left hand side is positive, the creation condition specifies that only the $+$ sign on the right hand side is applicable. That is, only charge of one species can be created in a given region. We will return to this point again at a later stage, accepting for the time being the $+$ sign in equation (4).

The relation (4) can be rewritten in a more familiar form:

$$R/(e^2/mc^2) \simeq N^{1/2} \quad (5)$$

stating that the 'radius' of the universe bears to the radius of the electron the ratio $N^{1/2} \sim 10^{40}$. This is one of the dimensionless numbers relating the fundamental constants.

The second relation follows when we take account of the relation between the Hubble's constant, the constant of gravitation G and the mean density ρ of matter in the universe. For the Friedmann model⁵ with the deceleration parameter $q_0 = 1$, or for the steady state model with the C field⁶,

$$\rho = 3H^2/4\pi G \quad (6)$$

Other models usually yield different coefficients of H^2/G on the right hand side, but they are of the same order as $3/4\pi$. Taking equation (6) to fix ideas, the mass of the matter in a sphere of radius R is around

$$4\pi R^3 \rho/3 \simeq c^3/HG \quad (7)$$

Observations of the Universe show that matter is largely made of equal numbers of electrons and protons at least in our neighbourhood. Taking M for the mass of a proton, the above relation gives an estimate of N :

$$NM \sim c^3/HG = c^2 R/G \quad (8)$$

If we now eliminate R between equations (5) and (8) we get

$$e^2/GMm \sim N^{1/2} \sim 10^{40} \quad (9)$$

The relations (5) and (9) are the core of the so-called Dirac relationships between fundamental constants. I now interpret these and discuss their astrophysical significance in the present picture by making the following points.

(1) Both relations express the overall dynamics of the Universe. The relation (5) arises from creation of charged leptons, the electrons and positrons in the cosmological framework. Although particles are created there is no overall loss of energy and momentum. Relation (8) may be looked upon either as an equipartition between the gravitational energy and the energy of expansion according to Einstein's equations or as a Machian relation determining the gravitational constant in terms of the matter content of the Universe^{7,8}.

(2) Although charge conservation is apparently violated when an electron is created, the violation is local, not global. The statistical fluctuation which requires the $+$ sign in equation (4) for a given region is balanced by an opposite fluctuation which favours the creation of the opposite charge in another region. Thus we will tend to have electrons created in one region, positrons in another. Also the process is stable in the sense that the creation of too many electrons in a given region will produce a local contribution to A_4 which will soon cancel the cosmological contribution and thus control the process.

(3) Relation (6) owes its origin to a statistical fluctuation and hence is not exact. That is, it is not possible to fix the coefficient of $N^{1/2}$ on the right hand side which will make it exact although the coefficient will be of the order unity. The relation (8) is exact in any given cosmological model. (The

~ sign appears because I have not specified the model in my discussion.) If I use the present determinations of c , H and G , I find that $N^{1/2} \sim 7 \times 10^{40}$ from equation (5) and $N^{1/2} \sim 1.3 \times 10^{40}$ from equation (8)—a reasonably good agreement.

(4) It may be possible to turn equation (4) round and argue that it implies creation of leptons of different masses as H varies or as we take into account the variation in fluctuation. This raises the interesting possibility that the electron mass is not fixed all over the Universe. This avenue is worth exploring in view of the puzzles about the QSO redshifts unearthed by recent observations^{9,10}.

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Production of Organic Molecules by Proton Irradiation

IN recent years there has been considerable interest in the production of complex organic molecules in primitive atmospheres. The emphasis of most of this work has been on mechanisms for prebiological synthesis of amino acids and related molecules, but the feasibility of producing organic chromophores which may account for the colours observed on planets and satellites in the outer Solar System by these same processes has become a topic of research effort in the past few years.

The early experiments¹⁻⁵ employed mixtures of methane and ammonia^{4,5}, sometimes with hydrogen¹ or water^{2,3}; and indeed amino acids (in the experiments with H₂O) and related organic molecules have been produced. More recently⁶⁻⁸, in attempts to simulate atmospheres likely to contain sulphur (those of Jupiter and primitive Earth), mixtures including hydrogen sulphide have been used. The results of these experiments were qualitatively similar to the non-sulphur ones, except for the production of free sulphur, alkyl sulphides and thio-amino acids. In one case involving a sulphur-free mixture a red polymeric material was reported⁴, and this is considered a tentative candidate for the red colouring agent on Jupiter.

With the exception of the work of Palm and Calvin², in which energetic electrons were used, one of two types of energy sources was employed: electrical discharges^{1,3-5} and ultraviolet light from a mercury lamp⁶⁻⁸. Electrical discharges may be initiated by strong atmospheric turbulence (similar to the familiar electrical storms) in the primitive atmospheres, and there is a significant amount of ultraviolet flux in the solar radiation. There is another source of energy, however, that is becoming increasingly worthy of consideration: energetic protons in the solar wind and in planetary radiation belts. Considerable attention has been given to the structure and

composition of the radiation belts around Jupiter and the proton (and electron) flux is believed to be far from insignificant⁹. The satellites of the outer planets certainly are exposed to the solar wind, and two of these, Io and Titan, are unusually red¹⁰. Titan is especially interesting as it is known to possess an atmosphere of at least H₂ and CH₄ (ref. 11). Also, protons can bombard a planetary atmosphere directly from discharges in the radiation belts. It is possible, then, for proton bombardment to have a measurable effect on the organic chemistry of primitive planetary atmospheres.

The following preliminary experiments were carried out to investigate the effectiveness of proton irradiation for producing organic chromophores. The source of the 2 MeV protons used in the experiments was a model FN tandem Van de Graaff accelerator of the physics department at Stony Brook. The beams used ranged from 870 nA (5.44×10^{12} protons cm⁻² s⁻¹ over 1/3 cm diameter) to 220 nA (1.35×10^{12} protons cm⁻² s⁻¹). Based on the nominal model⁹ for the flux in Jupiter's radiation belt at six Jupiter radii from the planet (approximately the distance of Io), these beams correspond to 1.72 and 0.43 Earth years at Jupiter per second of exposure. Thus, for typical exposures of 3 and 6 h respectively, the total proton fluences are equivalent to those of 1.9×10^5 yr and 9.2×10^4 yr at Jupiter.

The gas cells used were hollow aluminium cylinders, each with a gas volume of approximately 125 cm³. Attached to both ends of each cell were infrasil quartz windows, enabling visual inspection of any reaction products and gas phase spectroscopy from 0.2 to 4 μ m. A very thin pure nickel foil window was attached over a hole in the centre of each cell, perpendicular to the cylinder axis, to allow entry of the proton beam into the cell without loss of gas. The cells were evacuated to less than 10^{-3} mm Hg, filled with the appropriate gas mixtures (see below) and stored in a helium atmosphere to prevent contamination by air. The exposures were accomplished by placing the cells in a large target chamber, evacuating the chamber to 5×10^{-5} mm Hg, and running the beam into the cell for the desired length of time. The beam was stopped at the rear wall of the cell with approximately 1/2 MeV of the energy lost in the nickel window and 1 MeV deposited in the gas.

Five gas mixtures were used in this first series of experiments, none of which contained water. The first cell contained 50 cm³ CH₄ and 51 cm³ NH₃ at a total pressure of 615 mm Hg, and was exposed for 3 h at 870 nA. High resolution mass spectrometry of the gas phase indicated the presence of C₂H₂, C₂H₄, C₂H₆ and their methyl homologues, as well as C₄H₂ (diacetylene), CH₃CN, and residual CH₄ and NH₃. The orange-brown oily liquid produced was analysed by computer assisted gas chromatography-mass spectrometry^{12,13}, which indicated the presence of the expected alkyl hydrocarbons and alkyl amines. Some unexpected and interesting products were the tricyclic alkyl amines, hexamethylene tetramine (C₆H₁₂N₄) and its methyl and dimethyl homologues.

The second experiment was essentially the same as the first, except that the beam intensity was cut by a factor of four and the exposure was twice as long, resulting in the reduction of the total dose by half. The results of this run were similar to those of the first, except that the lower molecular weight molecules were enriched relative to the higher molecular weight ones. This indicates an 'evolutionary' sequence from low molecular weight to higher molecular weight, but further work will be done to confirm this.

A 1 : 1 : 1 mixture of CH₄ : NH₃ : H₂S at a total pressure of 605 mm Hg was used in the third experiment. Before the irradiation, a thin white haze (NH₄SH ?) was observed inside the cells, but it was not seen after the exposure. This seems to be analogous to the white residue noticed by Sagan and Khare⁸ in one of their experiments. The gas phase contained several simple hydrocarbons, saturated and unsaturated, along with CH₃CN and (CH₃)₂S. The gas chromatogram of the

liquid, which was much yellower than in the previous cases, indicated the presence of a series of alkyl di and tri-sulphides, starting with $(\text{CH}_3)_2\text{S}_3$ and increasing in alkyl chain length, as deduced from the mass spectra of the various fractions. The yellow colour was found to be free polymeric sulphur, predominantly S_8 . This is a very interesting result, as free sulphur (and subsequent ammonium polysulphides) have been proposed to account for the yellow and yellow-brown coloured layers on Jupiter¹⁴. Noticeably absent were the cyclic amines produced in the $\text{CH}_4:\text{NH}_3$ experiments; the exposure here was a relatively short one and the result may reflect the relative reactivities of H_2S and NH_3 .

In experiment four, a mixture of CH_4 with nitrogen was used instead of CH_4 and NH_3 . The gas phase contained the same species as in the NH_3 cases but the liquid, similar in appearance to the others, contained a much larger proportion of alkyl amines relative to the cyclic amines.

The last cell, containing only CH_4 , was run as a check on possible contributions of the apparatus itself to the final irradiation products. The gas phase again contained simple hydrocarbons, but also a trace of NH_3 , caused by the nitrogen remaining in the cell after pumping and filling. The clear non-viscous liquid formed contained a mixture of higher molecular weight hydrocarbons, both saturated and unsaturated. No traces of any compounds containing nitrogen or sulphur were found.

It is obvious from the above results that energetic protons can be an effective energy source for the formation of complex molecules from simple ones. Subsequent decomposition of the products is not as serious a problem as might be believed as there are likely to be processes, such as convection and condensation, in the atmospheres for removing the molecules from the reactive zone, and there were found to be fair amounts of the liquids formed on the rear windows of the cells in the centres of the proton beams. In spite of the extreme energies involved (2 MeV) as compared to typical bond energies, there seems to be some sensitivity to different initial reactants, as different results were obtained for the $\text{CH}_4:\text{NH}_3$ and CH_4N_2 mixtures, both containing only C, N and H. With the exception of the sulphide compounds none of the molecules that were identified are coloured; however, the colouring agents could be contained in the unresolvable fractions. It should be noted that no coloured compounds were produced in the pure CH_4 experiment; the presence of N or S seems to be necessary for their formation. Future experiments are being designed to probe this and other aspects relative to the outer Solar System colouring problem.

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Particle Formation from $\text{NH}_3\text{-SO}_2\text{-H}_2\text{O}$ -Air Gas Phase Reactions

RECENT articles on the formation of atmospheric aerosols from $\text{NH}_3\text{-SO}_2\text{-H}_2\text{O}$ gas phase reactions^{1,2} have considered only those products formed between the anhydrous gases to give 2:1 and 1:1 ($\text{NH}_3:\text{SO}_2$) compounds. I wish to draw attention to my work³ on the reaction between these gases at partial vapour pressures in air of 50 to 500 N m^{-2} . Solid products were formed only in the presence of water vapour at 20° C. Analyses showed that the compounds formed were either ammonium sulphite (with excess NH_3) or ammonium pyrosulphite (with excess SO_2). Vapour pressure measurements at 0 to 23° C indicated that the extrapolated vapour pressures of NH_3 and SO_2 above the sulphite in dry air at -70° C would be about 1×10^{-4} and 5×10^{-5} N m^{-2} respectively. With 50% saturation of the air with water vapour at -70° C, however, these vapour pressures would be depressed to 8×10^{-6} and 4×10^{-6} N m^{-2} respectively, that is, to concentrations approximately equal to those reported for the 2:1 compound. The partial vapour pressures of the pyrosulphite under similar conditions would be 3.3×10^{-5} N m^{-2} for both gases. At -52° C the vapour pressures of these gases in dry air would be identical for both the 2:1 compound and ammonium sulphite.

It seems, therefore, that there will be competition between the formation of the 2:1 compound and ammonium sulphite in the stratosphere except under completely anhydrous conditions.

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Thorium Determination in Glasses using Fission Track Technique

WE have applied the fission track technique to the determination of thorium in glasses. Neutron-induced fission occurs for thorium and uranium, the isotopes ^{232}Th , ^{235}U and ^{238}U fissioning with fast neutrons, and ^{235}U with thermal neutrons. Two irradiations are performed. The first irradiation is with predominantly thermal neutrons for determination of the uranium content (comparing to a standard and knowing the isotopic ratio of uranium) and the second irradiation is with predominantly fast neutrons which gives the uranium and thorium contents combined. The 'track excess' from the

second irradiation, when the unknown is again compared to a uranium standard, is attributed to thorium fission.

Consideration of several factors is important for evaluation of a standard. Knowledge of (1) the fission cross sections for the various isotopes as a function of neutron energy¹, (2) the neutron energy spectrum for the reactor facility² and (3) the isotopic concentrations enables the percentage of tracks contributed by any one isotope to be calculated. The total number of fissions produced ($\Sigma_f \Phi_f$) from a mixed neutron energy spectrum, where the fast neutron energy spectrum is taken between 1 and 10 MeV and the thermal neutron induced fission of only ^{235}U is considered, is given by

$$\Sigma_f \Phi_f = N^{232} \int_1^{10} \sigma_f^{232}(E) \phi_f(E) dE + N^{238} \int_1^{10} \sigma_f^{238}(E) \phi_f(E) dE + N^{235} \int_1^{10} \sigma_f^{235}(E) \phi_f(E) dE + N^{235} \sigma_f^{235} \phi_t$$

where N is the atom density of an isotope; $\sigma_f(E)$, the fast fission cross section of a specific isotope as a function of energy; $\phi_f(E)$, fast neutron flux as a function of energy; dE , energy interval; σ_t , the cross section for thermal fission of ^{235}U ; and ϕ_t , the thermal neutron flux.

tional to the density of the material through which they pass⁶. Therefore, an additional correction was made for the differences in densities of the standard glass and the unknown glasses. As expected, the error increases with a lower Th/U ratio. For glass K2a with a Th/U ratio of ~ 1 , our result was 20% greater than the value reported by Lovering. The fission of ^{235}U is still a major contributing factor, however, and by reducing this contribution by cadmium shielding, for example, or in a facility with a greater fast flux component, the error would be reduced.

This particular technique can easily be adapted for Th determination in minerals where the natural U isotopic ratio can be assumed, and would permit data to be acquired that would be more accurate than previously obtained by a neutron induced fission track method⁷. In addition, using this fission track technique for determining thorium and uranium avoids the uncertainties which result from beam current heating of the sample or observation efficiency differences when either α or other high energy particles are used to induce fission of thorium^{8,9}.

Part of this work was carried out under the supervision of the US Army's ECOM Night Vision Laboratory and TROS-

Table 1 Comparison of Fission Tracks and Other Techniques for Determination of Thorium

Sample	Th (p.p.m. by weight)*	U (p.p.m. by weight)	U isotopic ratio 8/5	Th (other method, p.p.m. by weight)	Density (g cm ⁻³)
La 1	87 ± 9	0.015 ± 0.002 †	Natural	76 ‡	3.60
La 2	265 ± 33	— §	Natural	—	3.56
La 3	466 ± 56	0.015 ± 0.002 † ‡	Natural	452 ‡	3.58
La 4	712 ± 60	— §	Natural	—	3.61
La 5	920 ± 65	— §	Natural	—	3.55
T-1	31.5 ± 1.0	0.046 †	189	31 ‡	2.28
				40.6 ± 0.8 **	
K2a	0.42 ± 0.04	0.345 ± 0.083 ¶ ¶	Natural	0.352 ± 0.008 ¶	2.03
614 SRM		0.823 † ‡	387	0.746 ‡	2.55

* Errors are one standard deviation based on track counting statistics.

† Nuclear track determination.

‡ Isotopic dilution mass spectrometry (all measurements by W. R. Shields *et al.* ± 0.25% standard deviation).

§ Since all the lanthanum glasses were made from the same base melt, it was assumed that the U content did not vary for the series of glasses.

¶ X-ray fluorescence.

¶¶ Neutron activation analysis.

** Using α irradiation, see ref. 9.

Therefore, from this equation and with our irradiation conditions with predominantly fast neutrons³, and for a material containing the natural isotopic ratio of uranium ($^{238}\text{U}/^{235}\text{U} = 137.8$), a Th/U ratio of 1,000 would mean that 80% of the tracks are from Th fission; a Th/U ratio of 4 means that only about 3% of the tracks are from Th fission. This low percentage approaches the limit of error imposed by track counting statistics. In our experiments, the standard used was NBS SRM 614 (ref. 4) where Th/U = 0.906 and $^{235}\text{U} = 0.2792$ atomic % abundant. Even with the depleted ^{235}U , this served well as a uranium standard in the fast neutron facility and only 1.5% of the tracks observed in the detector were from thorium fission.

The samples and standard were mounted in epoxy and polished; an external detector was then affixed. Mica was preferred for use as an external detector because the temperatures attained during the second irradiation with fast neutrons would partially anneal the tracks in a plastic detector.

The glasses used in this study consisted of five lanthanum-based glasses ($\sim 48\% \text{La}_2\text{O}_3$) used as thorium standards by the US Army Night Vision Laboratory at Fort Belvoir, Virginia. Thorium glass T-1 described by Schreurs and others⁵, and glass K2a described by Lovering (personal communication). The fission track results compared to Th determination by other methods (where data are available) are shown in Table 1. It was assumed that the range of fission fragments is propor-

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NaOH Etch of Fission Tracks in a Soda-Lime-Silica Glass

It is generally known that fission tracks intersecting the surface of man-made soda-lime-silica glasses can be etched with HF at room temperature^{1,2}. In this technique the acid attacks the tracks preferentially so that they become visible in the microscope before the glass itself is destroyed. An NaOH etch at 60° C has been reported for phosphate glasses³.

I wish to report a glass which I believe to be a soda-lime-silica glass that is etched by NaOH rather than HF. The material consists of numerous glass beads of a recognisable type known as 'trade wind beads' found in archaeological sites of the past millennium in eastern and southern Africa. They are consistently similar in appearance and chemical composition. Their composition is partly reported in Table 1 and abundant further information is available elsewhere⁴.

Table 1 Partial Composition of 'Trade Wind Beads'

Element	Concentration	Method	No. of samples
Na	15.0 ± 1.5%	NAA	133
Ca	4.4 ± 1.9%	NAA	133
K	2.3 ± 1.4%	NAA	133
Pb	~0.01–7.0%	XRF	~200
P	~0.1%	ES	2
U	112 ± 55 p.p.m.	NAA	133
(SiO ₂)	~56%	XRF	1)

NAA, neutron activation analysis; XRF, X-ray fluorescence analysis; ES, emission spectroscopic analysis.

The beads are thought to contain fossil fission tracks useful for dating. In an attempt to etch such tracks, experiments were carried out on artificially produced fission tracks in 'trade wind beads'. HF at various concentrations at room temperature attacked the glass without etching the tracks preferentially. Successful etching was accomplished by refluxing for 7 min in NaOH. The reflux solution was made by dissolving 12 g NaOH in 16 g water.

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BIOLOGICAL SCIENCES

Proximal Myopathy induced by 5-HT-Imipramine simulates Duchenne Dystrophy

It has recently been observed that patients with Duchenne muscular dystrophy (DMD) have a significant defect in the uptake of serotonin (5-HT) into platelets¹, raising the possibility that a defect in the metabolism of biogenic amines may be important in the pathogenesis of the skeletal muscle lesions of this disorder. If monoamines such as

5-HT or norepinephrine (NE) which are dependent on the intracellular enzyme, monoamine oxidase (MAO), have a diminished ability to reach this site of metabolism, increased tissue levels of these compounds could result and produce skeletal muscle damage. The effect of such a defect would be enhanced in skeletal muscle as catechol-O-methyltransferase, the alternative pathway for metabolism of biogenic amines, is absent from this tissue². Support for this hypothesis of skeletal muscle damage is found in the observation that skeletal muscle from biopsies of Duchenne dystrophy patients has shown the presence of an abnormal intrafibrillar fluorescence (Falck-Hillarp technique) presumed to be a monoamine-like substance³. Furthermore, injection of the MAO inhibitor, pargyline, results in a myopathy with evidence for accumulation of monoamines⁴. The pargyline induced myopathy, however, has serious limitations as a model for Duchenne dystrophy, as the lesions are limited to the soleus muscle and, furthermore, MAO activity has been found to be normal in patients with DMD (ref. 1).

Here we report the findings of an experimental myopathy using 5-HT in combination with imipramine, a pharmacological agent blocking uptake of biogenic amines into platelets⁵ and thereby producing a defect in biogenic amine metabolism closely simulating that observed in patients with DMD. The results of this study are extremely important in two ways. First, the very typical early and mid-stage histological lesions of DMD were reproduced and, second, this study represents the first demonstration of an experimental animal model with skeletal muscle lesions predominating in the proximal musculature, as is so characteristic of the clinical disease.

Fifty male Osborne-Mendel and Sprague-Dawley rats weighing 135 to 150 g were used. Fresh frozen sections of soleus, gastrocnemius, anterior tibialis, extensor digitorum longus, quadriceps, iliopsoas and deltoid muscles were stained by modified trichrome, haematoxylin and eosin, reduced nicotinamide dinucleotide-tetrazolium reductase and myofibrillar adenosine triphosphatase⁶. At the beginning of the study all rats were injected intraperitoneally with imipramine, 10 mg kg⁻¹, for 3 d. On the fourth day the animals received 5-HT, i.p., 100 mg kg⁻¹. Each week two rats were killed 4 d after the injection of 5-HT, the remaining rats being injected with the same dosage schedule for a total of 11 weeks.

Skeletal muscle lesions were seen in all rats examined and increased in severity as the number of injections in each rat increased. The distribution of the histological lesions in these animals was quite striking as the lesions were very severe in the quadriceps, moderately severe in iliopsoas and occasionally present in the soleus. In the gastrocnemius, anterior tibialis and extensor digitorum longus, only rare skeletal muscle pathology was encountered. The earliest lesions consisted of necrosis and phagocytosis and regeneration of individual muscle fibres or small groups of muscle fibres surrounded by a field of normal muscle (Figs 1 and 2). This is identical to the early lesions of DMD (ref. 7). In animals receiving multiple injections, the lesions showed signs of chronicity manifested by a marked variability in fibre size, internal sarcolemmal nuclei and proliferation of connective tissue surrounding individual muscle fibres (Fig. 3). This is characteristic of the mid-stage histological lesions of DMD (ref. 7). No abnormalities were seen in the intramuscular nerves and the intramuscular arteries showed no signs of occlusion, although, rarely, they were surrounded by a small round cell infiltrate, similar to that described in DMD (ref. 8).

Control animals included those injected i.p. with imipramine 10 mg kg⁻¹ as above but without follow-up injections of 5-HT. Another group of animals was injected i.p. daily for 7 d with imipramine, 25 and 50 mg kg⁻¹. A third group of controls included those injected with single

doses of 5-HT, 100 mg kg⁻¹. Only rare scattered fibres undergoing necrosis and phagocytosis were seen in the animals injected with imipramine, 50 mg kg⁻¹ and 5-HT, 100 mg kg⁻¹.

We feel that this animal model of Duchenne dystrophy is extremely important because not only have the highly characteristic histological skeletal muscle lesions been reproduced but of possibly greater significance is the fact that the proximal muscles were found to be the principal site of lesion predilection. These abnormalities were the result of injections of 5-HT in combination with a pharmacological agent, imipramine, which reproduces an abnormality in biogenic amine metabolism similar to that found in patients with DMD. This study adds significance to the previously described transport defect of 5-HT (ref. 1) in DMD and opens many new avenues of investigation in a disease which has remained an enigma since its description. Furthermore, this 5-HT-imipramine induced myopathy provides a suitable animal model for future study which is superior to others previously described using arterial embolisation⁸ or aortic ligation in combination with 5-HT or NE (ref. 7). These models, requiring surgical manipulation, failed to show a predilection of skeletal muscle pathology in a proximal distribution.

The pathogenesis of the proximal muscle lesions produced in this animal model remains to be elucidated. It is unclear at this time whether the lesions are the result of functional ischaemia to the skeletal muscle or are the result of direct myotoxicity. It also remains to be established whether

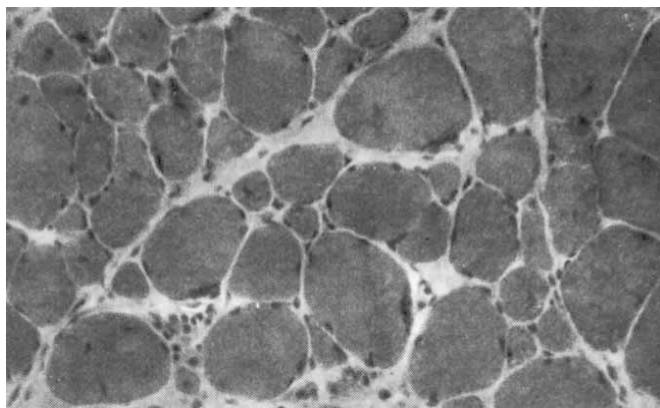


Fig. 3 A group of muscle fibres of variable size, many with internal nuclei, and proliferation of endomysial connective tissue between individual fibres. ($\times 194$.)

or not these lesions are dependent on an intact nerve supply as are those induced by the MAO inhibitor, pargyline. Studies are currently under way to answer these and other questions.

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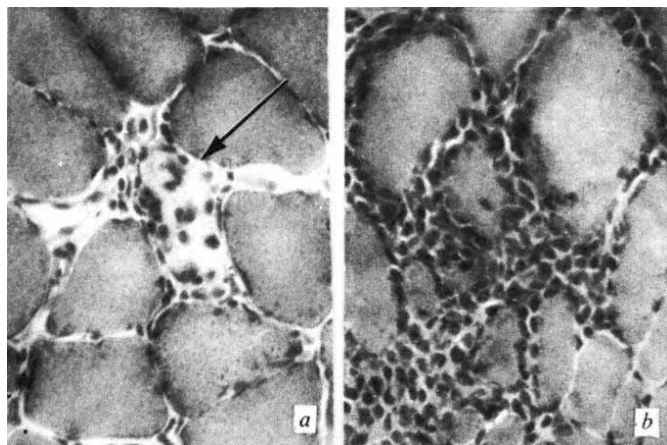


Fig. 1 a, Single muscle fibre undergoing necrosis and phagocytosis (arrow) in soleus. ($\times 150$.) b, Small group of muscle fibres undergoing necrosis and phagocytosis in quadriceps. ($\times 280$.)



Fig. 2 A group of small regenerating muscle fibres in quadriceps surrounded by normal fibres. Note the enlarged central nuclei characteristic of regeneration. ($\times 155$.)

Dose Dependence of Antigen Binding to B and T Lymphocytes

SPECIFIC precursor cells, as lymphocytes binding ¹²⁵I-labelled antigens, can be detected by autoradiography (see refs 1 to 3 for reviews). Classification of such antigen binding cells (ABC) into T and B cells gave equivocal results. Cell populations enriched in B cells (by treatment with anti- θ serum and complement) or in T cells (by passage through anti-immunoglobulin columns) indicated that some of the ABC were indeed T cells, but that they bound far less antigen than did B cells⁴⁻⁷. Using a direct approach by treating lymphocytes in sequence with ¹²⁵I-antigen (*Maia squinado* haemocyanin: MSH, or bovine serum albumin) followed by fluorochrome-labelled anti-immunoglobulin, anti-B, anti-T, or anti- θ reagents, Lamelin *et al.*⁸ did not detect significant numbers of T ABC, while Roelants *et al.*⁹ using the same antigen MSH and also the heavily iodinated (>50 atoms I per molecule) (Tyr, Glu)-Ala-Lys synthetic polypeptide (TIGAL) found that 20-30% of the ABC in primed mice were T lymphocytes and that they bound as much antigen

as B ABC. Joint experiments (J. P. Lamelin and G. E. Roelants, unpublished) showed that this discrepancy was due to the hundred-fold lower concentration of antigen used by Lamelin *et al.* The influence of antigenic concentration on the binding by B and T cells has been systematically investigated, with the following results.

Antigen binding, with detection of immunoglobulin or θ surface markers on the same cell, has been described⁹. Briefly, 20×10^6 spleen or lymph node cells were mixed with various amounts of ^{125}I -TIGAL (20 to $300 \mu\text{Ci } \mu\text{g}^{-1}$) in a final volume of 0.4 ml ice cold Eagle's Minimal Essential Medium, 10% foetal calf serum (FCS), 1.5 mM NaN_3 . In preliminary experiments cells were also exposed to antigen ($2.5 \mu\text{g ml}^{-1}$) at higher temperatures (23°C and 37°C) which only had a minimal effect on ABC frequency (10–20% increase of both B and T ABC). Further experiments were always carried out in the cold. After 45 min the cells were washed of excess antigen, incubated with either rhodamine-conjugated rabbit anti-mouse Ig or AKR anti- θ C3H immunoglobulin, washed, smeared on gelatine-coated slides and fixed for 5 min in absolute ethanol. Autoradiography was performed using Ilford K5 emulsion. The cells were observed under phase contrast at 100×6.3 -fold magnification with Leitz Orthoplan microscopes equipped with Osram HBO-200 mercury vapour lamps and Opak-Fluor vertical illuminators (E. Leitz GmbH, Wetzlar, Germany). B lymphocytes were identified as immunoglobulin positive or θ negative and T lymphocytes as immunoglobulin negative or θ positive. The validity of this classification has been discussed in detail⁹.

Lymph nodes or spleen cells from unprimed C3H/He mice (either sex, 2.5 to 5-month-old, Roche, Füllinsdorf, Switzerland) or spleen cells from primed mice ($100 \mu\text{g}$ TIGAL precipitated with alum and 10^9 killed *Bordetella pertussis* intraperitoneally 4 month previously) showed a plateau in the number of B and T ABC at antigen concentrations of about $2 \mu\text{g ml}^{-1}$. Cells pooled from 4–6 mice were used for each experiment. In accordance with reported results⁹ at the plateau the overall frequency of ABC 4 month after priming was not very different from that of unprimed mice (400×10^{-5} to 700×10^{-5}). Figure 1 shows, for example, the ABC frequency of normal lymph node cells exposed to concentrations of TIGAL ranging from 0.5 to $4 \mu\text{g ml}^{-1}$. Earlier experiments over the range of 2 to $64 \mu\text{g ml}^{-1}$, at two-fold steps, did not cover the lower limit of the curve but showed that the plateau extended over all concentrations above $2 \mu\text{g ml}^{-1}$, so the rise at $4 \mu\text{g}$ in Fig. 1 was not significant.

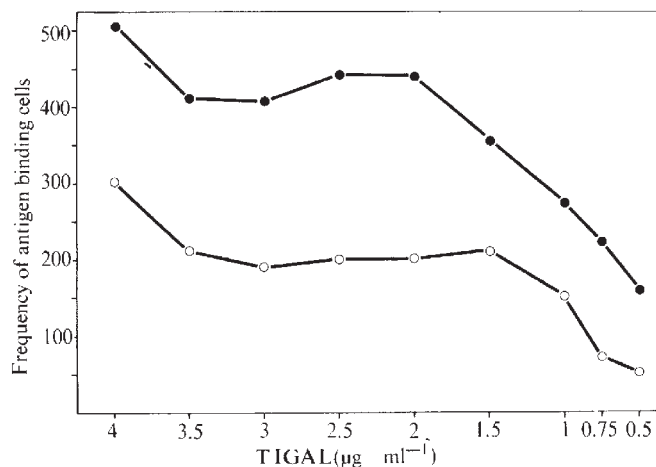


Fig. 1 Frequency of antigen binding B (●, $\times 10^{-5}$) and T (○, $\times 10^{-6}$) cells in a normal lymph node cell population at various concentrations of antigen (50,000 to 100,000 lymphocytes were examined per group).

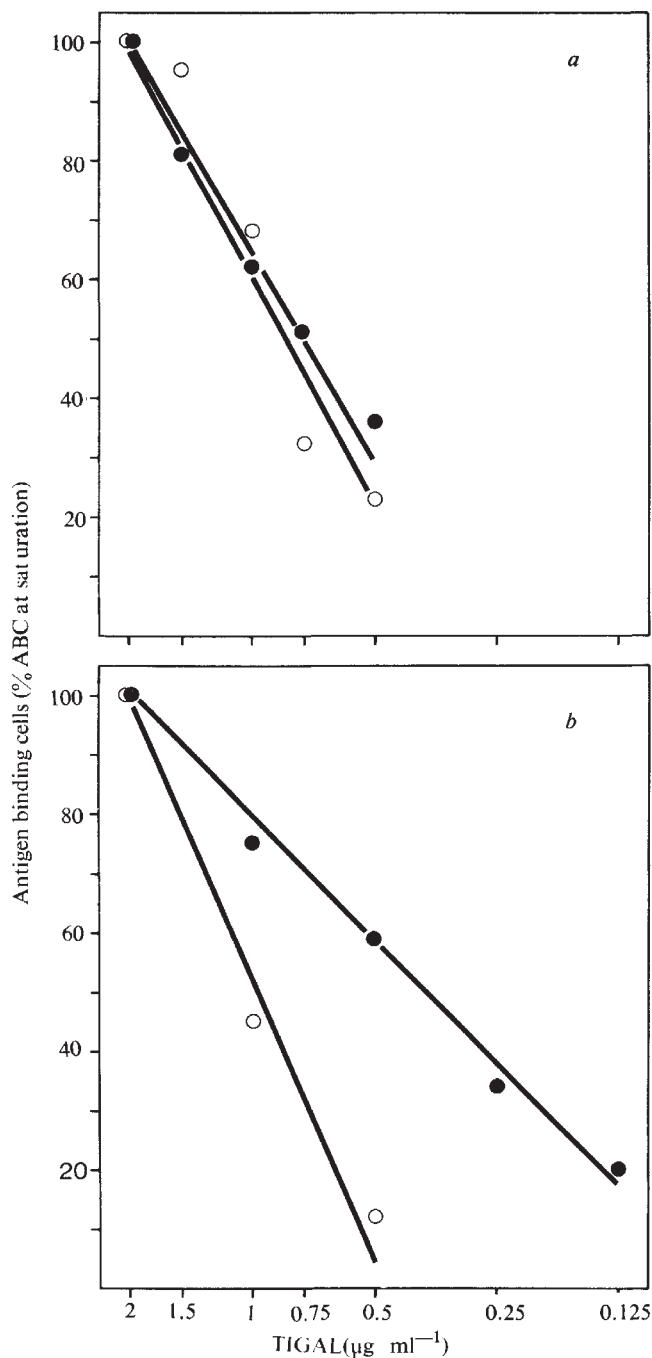


Fig. 2 Titration of, a, normal and, b, TIGAL-primed antigen binding cells at antigen doses below the plateau level (50,000 to 100,000 lymphocytes were examined per group). ●—●, B; ○—○, T lymphocytes.

Figure 2 shows the regression of B and T ABC on antigen concentrations below the saturation level. For unprimed cells the slopes are similar for B and T ABC; the slope of B ABC decreases after priming. This observation, in agreement with those of Möller and co-workers^{10,11} using hapten inhibition of rosette formation, indicates that the avidity of both T and B rosette-forming cells is similar in unprimed animals, whereas after priming the avidity of B cells is increased, but that of T cells remains unchanged.

The degree of labelling, as indicated by the number of grains, was similar for T and B cells at a TIGAL concentration of about $2 \mu\text{g ml}^{-1}$. Varying the concentration of antigen had a strikingly different effect on the degree of labelling of B or T ABC. As exemplified in Fig. 3 with a primed spleen cell population, the proportion of heavily

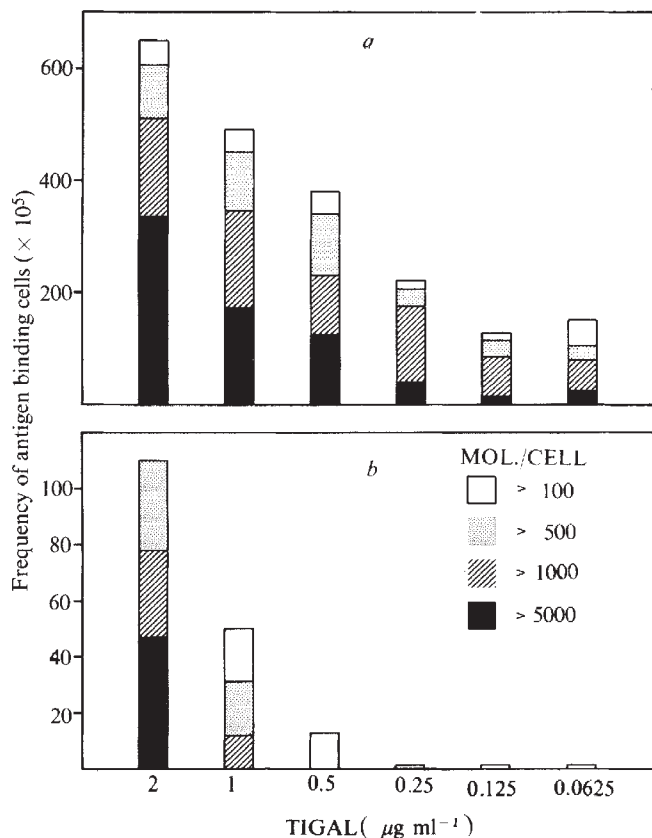


Fig. 3 Degree of labelling of TIGAL-primed antigen binding cells at various antigen doses. The ABC were distributed into four classes depending on their degree of labelling expressed as molecules per cell (see inset legend). a, B cells; b, T cells.

labelled B cells decreased when the concentrations of antigen were lowered, but even at the lowest concentrations used ($0.0625 \mu\text{g ml}^{-1}$) some heavily labelled B cells were still present. In sharp contrast all the heavily labelled T cells were lost by a mere two-fold dilution from the plateau ($1 \mu\text{g ml}^{-1}$). Unprimed cells behaved the same way, even though the overall proportion of T and B cells labelled was similar at different concentrations of antigen (see Fig. 2). Since the number of B ABC did not increase between 4 and $64 \mu\text{g TIGAL ml}^{-1}$, some B cells were saturated with as little as 100 molecules of antigen. T cells seemed to be more homogeneous in this respect since they all become heavily labelled at high concentrations of TIGAL. Whether all T ABC bind a maximal amount of antigen (about 50,000 molecules) at saturation cannot be decided from these experiments, due to overlapping and confluent grains above 500 per cell.

These observations render it mandatory to establish binding curves over a wide range of concentrations. Without this tedious process, no valid information about the number of B and especially T ABC can be obtained. Hämmerling *et al.*^{6,7} studying ABC for (T,G)-A-L (antigenically different from TIGAL) used concentrations of antigen between 0.5 and $1 \mu\text{g ml}^{-1}$ and detected only T ABC binding less than 500 molecules of antigen. Even with the far more potent immunogen TIGAL used here no heavily labelled T ABC are detected at those antigen concentrations. Binding curves for (T,G)-A-L are being determined and preliminary results indicate that a plateau is reached only for antigen concentrations as high as $25 \mu\text{g ml}^{-1}$.

As shown by the saturation experiments, T cells possess a number of receptors for antigen comparable to that of B cells. The effect of antigen dilution on the distribution of label density is, however, far more pronounced for T than for B ABC, indicating that the average avidity for antigen

is both lower and more uniform. Whether this is due to an inherent property of the receptor or is due to the nature of the T cell membrane remains to be investigated. The fact that even primed B ABC have widely varying numbers of receptors suggests that the high avidity of primed B ABC may be due not only to selection of cells with higher affinity receptors but also to 'maturation' of cells acquiring a larger number of receptors. Neither selection nor maturation could be discovered for T ABC.

The titration curves of this study do not argue either for or against the immunoglobulin nature of the T-cell receptor. Experiments approaching this problem in a direct way are presented in a companion paper¹².

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Active Synthesis of Immunoglobulin Receptors for Antigen by T Lymphocytes

WHEN antigen binding cells (ABC) were studied by combined autoradiography and immunofluorescence it was found that antigen binding to both B and T ABC could be inhibited by anti-immunoglobulin (anti-Ig) reagents¹. Moreover, receptors for antigen could be redistributed in caps in a similar way for B and T ABC using non-inhibiting doses of anti-Ig reagents¹. Since membrane components are known to redistribute independently of each other²⁻⁵ it was concluded from the inhibition and capping experiments that the receptor for antigen on T lymphocytes was immunoglobulin. The fact that T ABC are always a small minority of the total T cell population argues against the possibility that their receptors are passively acquired as cytophilic antibody. We present evidence confirming that T cells have receptors for antigen which are immunoglobulins and showing, in addition, that they are actively synthesised by the cells carrying them.

Both the redistribution of surface immunoglobulin after interaction with anti-Ig reagents, and the combined autoradiographic immunofluorescent technique of estimating antigen binding and demonstrating immunoglobulin or θ surface markers on the same cell, have been described previously^{1,3}. Our present experimental scheme is set out in Fig. 1. Lymph node cells (80 to 85% T cells) from four to six unprimed C3H/He mice per experiment (either sex, 2.5 to 5 months old, Roche, Füllinsdorf, Switzerland) were exposed

to a polyvalent rabbit-anti-mouse immunoglobulin reagent labelled with fluorescein isothiocyanate (FITC-RiG- α -MiG) and incubated under conditions allowing polar redistribution (capping) and endocytosis of cytoplasmic membrane immunoglobulin. In some experiments a second layer of unlabelled sheep anti-rabbit L chain reagent (Sser- α -RL)³ was added to ensure complete clearing of surface Ig. After various incubation times, samples of cells were taken and exposed, under non-capping conditions, to radioactively labelled heavily iodinated (Tyr, Glu)-Ala-Lys synthetic polypeptide (> 50 atoms I per molecule) (¹²⁵I-TIGAL) at saturating doses for detection of ABC⁶. The cells were then divided into two parts under non-capping conditions: one was stained with rabbit anti-mouse immunoglobulin (IgG) reagent labelled with tetramethylrhodamine isothiocyanate (TRITC-RiG- α -MiG)³, the other with rhodamine-labelled AKR anti- θ -C3H IgG (TRITC-anti- θ)^{1,7}. As a control, cells were first incubated with a rabbit IgG antibody directed against tobacco mosaic virus (FITC-RiG- α -TMV)³ instead of FITC-RiG- α -MiG; subsequently these cells went through the same steps as the experimental group.

- (1) Normal lymph node cells in RPMI 1640 medium 0.03 M HEPES, 10% foetal calf serum (FCS), 2 mM glutamine, penicillin-streptomycin, pH 7.2

+ FITC-RiG- α -MiG 250 μ g ml⁻¹ + FITC-RiG- α -TMV (control) 250 μ g ml⁻¹
 0° C, 10 min } 50 $\times$ 10⁶ cells ml⁻¹,
 37° C, 50 min } medium without NaN₃
 wash 3 $\times$

- (2) (Optional) + Sser- α -RL 1/40 final dilution
 37° C, 15 min, 50 $\times$ 10⁶ cells ml⁻¹,
 medium without NaN₃
 wash 3 $\times$

- (3) Incubate at 37° C, for variable lengths of time, 0.5 $\times$ 10⁶ cells ml⁻¹, medium without NaN₃, 4 ml in Falcon Tissue Culture Flasks 3012.

wash 2 $\times$ in medium with NaN₃ (1.5 mM), 0° C.

- (4) + ¹²⁵I-TIGAL (200–468 μ Ci μ g⁻¹) 2.5 μ g 25 $\times$ 10⁶ cells ml⁻¹
 0° C, 30 min, medium with NaN₃ (1.5 mM)
 wash through FCS gradients

- (5) + TRITC-RiG- α -MiG 250 μ g ml⁻¹ + TRITC-anti- θ 250 μ g ml⁻¹
 0° C 30 min,
 50 $\times$ 10⁶ cells ml⁻¹,
 medium with NaN₃ (1.5 mM)

- (6) Wash 3 $\times$, smear on gelatine coated slides, fix in absolute ethanol (5 min), process for autoradiography (Ilford K5 emulsion).

Fig. 1 Basic experimental procedure.

Characterisation of the various reagents used has been described^{1,3,7}. The main specificity controls were as follows. TRITC-anti- θ stains the expected proportion of C3H mouse lymph node, spleen or thymus lymphocytes but stains neither AKR mouse thymocytes nor nude mouse spleen cells. Its capacity to stain C3H thymocytes or spleen cells is completely removed by absorption with C3H mouse brain. The capacity of TRITC-RiG- α -MiG to stain B cells, to block or to induce polar redistribution of receptors for antigen on T and B cells is removed by absorption with unsolubilised IgG myeloma.

We used the FITC conjugate of the same RiG- α -MiG preparation. The capacity of both TRITC and FITC-RiG- α -MiG to stain B lymphocytes is absorbed by passage on electrophoretically purified IgG from normal mouse serum coupled to Sepharose. Anti-lymphocyte serum does not inhibit antigen binding to B and T lymphocytes and does not redistribute in polar caps, indicating that the activity of our reagents is not simply due to anti-membrane contamination.

The basic experiment was repeated five times, always using normal lymph node cells to exclude cytophilic anti-TIGAL antibody. The findings were reproducible and a sample is given in Fig. 2 (see also Table 1).

First treatment	Cell type	Start	10min	1h	6–18h
FITC-RiG- α -MiG	B				
	T				
FITC-RiG- α -TMV	B				
	T				

○ FITC - RiG - α -MiG ≡ TRITC - anti- θ
 ● TRITC - RiG - α -MiG ≡ ¹²⁵I - TIGAL

Fig. 2 Capping and resynthesis of immunoglobulin receptors for antigen on T and B lymphocytes. For experimental protocol see Fig. 1 and text.

FITC-RiG- α -MiG induced capping and endocytosis of cell surface Ig as detected on B cells by fluorescence microscopy (Leitz Orthoplan microscopes equipped with Osram HBO-200 mercury vapour lamps and Opak-Fluor vertical illuminators; 100 $\times$ 6.3 or 100 $\times$ 1.25 $\times$ 6.3 magnifications^{1,3}). Endocytosis was complete in about 1 h. At this point the cells were no longer stained by the subsequent treatment with TRITC-RiG- α -MiG. As described before¹, during the capping treatment both B and T ABC at first showed ¹²⁵I-TIGAL radioactive grains in caps, superimposed on the Ig caps in the case of B ABC, but after about 1 h, neither B nor T ABC were detected. In three experiments, ABC and surface immunoglobulin started to reappear about 3 h after capping and the receptor resynthesis was complete in about 6 h, in agreement with earlier observations³. In two experiments, however, complete resynthesis took 12–18 h. It is noteworthy that T ABC in each case followed the same kinetics of disappearance and reappearance as B ABC.

It would seem far-fetched that, in a population of normal lymph node cells, rare T cells (frequency 23–43 $\times$ 10⁻⁵) would bear at their surface enough passively adsorbed antibody to bind up to 50,000 molecules of TIGAL and even less likely that during the *in vitro* incubation after capping, TIGAL-specific B cells (frequency 54 $\times$ 10⁻⁵ to 108 $\times$ 10⁻⁵) would have secreted enough anti-TIGAL antibody which would bind only to those rare T ABC to bring their number back to their initial level. It could still be argued that the antigen-specific T-cell receptors were associated with some passively acquired Ig, were co-capped with it by the anti-Ig treatment, and were

Table 1 Active Synthesis of Ig Receptors for Antigen by B and T Lymphocytes

	Proportion of B cells in total population	ABC frequency $\times 10^{-5}$ *		Groups compared	Statistical analysis P value†	
		B	T		B	T
1 Untreated population	17%	69	28	1:2	<0.0005	<0.01
2 1 h after first capping with RIGG- α -MIGG	17%	0	0‡	2:3	<0.001	<0.005
3 18 h after first capping with RIGG- α -MIGG	9%	25	21	3:4	<0.001	<0.005
4 1 h after second capping with RIGG- α -MIGG	7%	0	0	1:3	<0.025	>0.60
					>0.40§	
5 1 h after RIGG- α -TVM	17%	84	47	1:5	>0.50	>0.30
6 18 h after RIGG- α -TVM	10%	23	21	1:6	<0.025	>0.50
					>0.50§	

* 50,000 to 75,000 lymphocytes were examined per group.

† Based on χ^2 test.

‡ One cell was found with ~thirty grains still in a small cap.

§ When corrections were made for the decrease in the overall proportion of B cells, which was found in two experiments out of five.

later resynthesised. Although there is no precedent for such co-capping, we sought formal proof that the antigen binding receptors which reappeared on B and T ABC were indeed immunoglobulin.

Two experiments were therefore extended by taking samples of cells known to have capped and resynthesised and exposing these cells once again to FITC-RIGG- α -MIGG. They were then, as before, put in capping conditions and later exposed, under non-capping conditions, to 125 I-TIGAL, followed by TRITC-RIGG- α -MIGG. Results of one complete experiment are given in Table 1: receptors for antigen on both B and T ABC which had been resynthesised after capping were cleared again by a second treatment with anti-Ig reagents. Samples of cells were examined before the total clearance of surface receptors by the second FITC-RIGG- α -MIGG treatment and showed that this clearance was also due to capping.

These experiments demonstrate the active synthesis of immunoglobulin receptors for antigen by T lymphocytes. The nature of these receptors in terms of known Ig classes remains to be investigated. It is perplexing that these receptors are not readily visualised using conventional fluorescent anti-Ig reagents. This may be due to their location in the T cell membrane; for instance, only a small part of the Ig receptor molecule(s) may be accessible to rabbit anti-M IgG antibody. These parts may, however, be detectable by antibody present in anti-M Ig antisera raised in chicken (F. L., J. R. L. Pink and L. Du Pasquier, manuscript in preparation).

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Increase in Kallikrein Excretion during the Natriuresis produced by Arterial Infusion of Substance P

SUBSTANCE P was discovered by von Euler and Gaddum in 1931 in extracts of brain and small intestine¹ and its distribution and effects have recently been reviewed². Its structure has been identified³ and the undecapeptide has recently been synthesised⁴. Substance P is a potent stimulator of salivary secretion⁵; stimulation of the chorda-lingual nerve leads to increases in the production of saliva and blood flow through the salivary gland and is associated with the depletion of glandular kallikrein⁶. The presence of atropine blocks secretion but does not affect the increase in blood flow⁷ or kallikrein depletion⁸. In homogenised nervous tissue substance P is located in the fraction containing the synaptic vesicles⁹.

It seemed from these findings that substance P at the nerve endings might be released and lead to the activation or release of kallikrein. Kallikrein has been demonstrated in a variety of tissues which produce a liquid secretion such as salivary glands⁹, small intestine¹⁰, pancreas¹¹, sweat glands¹² and kidneys¹³. Urinary kallikrein resembles renal kallikrein¹⁴ and differs from plasma kallikrein¹⁵. In severe renal failure urinary kallikrein excretion is very high which also supports the view that it is of renal origin and not filtered from plasma¹⁶. Kallikrein excretion is closely related to sodium excretion in a variety of circumstances¹⁶⁻¹⁸. Nevertheless increased sodium excretion also occurs without an associated increase in kallikrein excretion when there is a sharp fall in mineralocorticoid activity. These two processes related to sodium excretion are clearly differentiated by the Na/K ratio in the urine¹⁹.

If 'substance P-ergic' nerves exist and promote the release of kallikrein, it might be expected that substance P infusion into the renal artery would lead to the release of kallikrein and a natriuresis. This has been tested in greyhound dogs under pentobarbital anaesthesia. Both ureters were catheterised and 0.9% saline was infused into the left renal artery at 1 ml min⁻¹. After several 10 min control collections of urine the infusion was changed to synthetic substance P (Beckman) in saline for three periods, then back to saline alone. Urinary sodium was measured by flame photometry. Kallikrein was assayed by determining the esterase activity of the enzyme in releasing tritiated methanol from a synthetic labelled substrate (*p*-tosyl-L-arginine-³H-methyl ester)²⁰. Pancreatic kallikrein (Bayer) was used as a standard in the assay and the results are given in esterase units, which are defined in Table 1.

In thirteen experiments on five dogs doses of 1, 10 and 100 ng substance P min⁻¹ were used, equivalent to 30-4,300 pg kg⁻¹ min⁻¹. The changes in sodium and kallikrein excretion, calculated as the differences between excretion in the experimental periods and the mean of the control periods immediately preceding and following the peptide infusion, are shown in the

Table 1 Increases in Sodium and Kallikrein Excretion in Response to Arterial Infusions of Substance P

Substance P dose (ng min ⁻¹)	Sodium excretion (μEq min ⁻¹)		P	Kallikrein excretion (mEU min ⁻¹)		P
	Control	Increase		Control	Increase	
1	38.8 ± 9.67 n (8)	11.7 ± 4.35 (12)	<0.025	411 ± 87 (8)	12 ± 27 (12)	NS
10	43.8 ± 9.64 n (10)	67.3 ± 14.73 (15)	<0.001	395 ± 98 (10)	127 ± 33 (15)	<0.005
100	55.0 ± 14.27 n (8)	128.9 ± 19.16 (12)	<0.001	443 ± 123 (8)	192 ± 46 (12)	<0.005

Means ± s.e.

n, Number of control or experimental periods (see text).

P is derived from a *t* test on paired values.EU, Esterase unit, equivalent to the hydrolysis of 4.8 μmol *p*-tosyl-L-arginine-methyl ester per h at pH 8.5 and 37° C.

table. The mean increases in sodium excretion were significant at each of the three doses. Mean kallikrein excretion increased at 1 ng min⁻¹ though not significantly, but at 10 and 100 ng min⁻¹ the increases were highly significant ($P < 0.005$). The changes in excretion of sodium and kallikrein, when correlated with the logarithm of the dose of substance P infused, showed a highly significant correlation: $r = 0.756$; $P < 0.01$ for sodium and $r = 0.735$; $P < 0.01$ for kallikrein.

The data on sodium excretion place substance P amongst the most potent natriuretic substances so far described. Its threshold dose (around 1.0 ng min⁻¹) is less than, and its maximal effect greater than, those of the prostaglandins E₁, E₂ and A₁ found in comparable studies on dogs²¹⁻²³. The concentration in the renal artery plasma which was natriuretic was approximately 0.1–1.0 ng ml⁻¹ which is well below the limit of detection of a recent radioimmunoassay for substance P (ref. 24).

A variety of vasodilators are natriuretic when infused into the renal artery, for example bradykinin²⁵, lysyl-bradykinin²⁶, dopamine²⁷ and acetylcholine²⁸. Substance P is also a vasodilator²⁹. Since substance P stimulates natriuresis, salivary secretion and nasal secretion and all these processes are related to an increase in kallikrein activity, it is possible that the vasodilator action is dependent upon the release of kallikrein. There is strong evidence that urinary kallikrein originates from the kidney¹⁴⁻¹⁶, being located principally in the cortex³⁰.

In view of the similarity between nervous stimulation and substance P infusion in their effects on stimulation of saliva flow and the related release of kallikrein and vasodilation in the salivary gland³⁻⁷, the release of kallikrein from the kidney might be a direct effect of the substance P.

The effect of substance P on the kidney is a further example of the relation which has been shown between kallikrein excretion and sodium excretion¹⁸⁻¹⁹ but in this case there is no associated expansion of body fluid volumes. The bradykinin released by kallikrein is known not only to be a vasodilator but also to increase vascular permeability. The natriuresis produced by the intra-renal release of kallikrein may be effected by vasodilator and permeability effects on the renal vasculature and tubules.

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Androgen-induced Sexual Differentiation of the Brain is Blocked by Inhibitors of DNA and RNA Synthesis

SEXUAL differentiation of gonadotrophin secretion in the rat is known to occur during the early postnatal period under the action of testicular androgens^{1,2}. Animals of either sex if exposed to androgen during this critical period develop the

typical male pattern of gonadotrophin secretion after puberty with failure of ovulation, small polyfollicular ovaries lacking corpora lutea and a constant cornified vaginal epithelium. Several studies have localised this effect to the brain^{3,4} and more particularly to the anterior hypothalamus⁵, but very little is yet known of the mechanism of this effect.

What is known of the action of androgens and oestrogens on their peripheral target tissues would suggest that the steroid enters the cell, is rapidly transported to the nucleus and, by an action on the genome, induces specific RNA and protein synthesis and later DNA synthesis and cell division⁶⁻⁸. Inhibitors of these processes might therefore be expected to provide some protection against the masculinising effects of androgen in the neonatal female rat. Attempts to achieve this with inhibitors of RNA and protein synthesis by Gorski and coworkers^{9,10} have so far produced only partial and/or temporary inhibition of the action of a low dose of androgen (30 µg of testosterone propionate, TP). Barnea and Lindner¹¹ gave direct intracerebral injections of DNA and protein synthesis inhibitors but failed to achieve any protection against the effects of 100 µg of TP. We therefore tested the effects of a variety of inhibitors of DNA, RNA and protein synthesis combined with three different dosages of TP and have obtained evidence of permanent and, in some cases, almost complete protection against the masculinising effects of the androgen.

Female Wistar rats were treated when 4 d old with 30, 80 or 200 µg of TP s.c. in oil and the inhibitors were administered by the same route in divided doses together with and 6 h after the TP. The inhibitors used were: hydroxyurea (OH-U), an inhibitor of DNA synthesis with no effect on overall RNA synthesis¹²; α -amanitin (AMAN), an inhibitor of nucleoplasmic or messenger-precursor RNA synthesis¹³; actinomycin D (ACT.D), at the low doses used in this study, a selective inhibitor of nucleolar or ribosomal RNA synthesis¹⁴; puromycin (PURO), a general inhibitor of protein synthesis, and fluorouracil (FU) which induces errors of translation and the synthesis of non-functional proteins. Cycloheximide was also tested at several doses but found to be too toxic for use in neonatal rats (compare refs 10 and 11). The progress of masculinisation was followed by daily vaginal smears for at least 3 weeks prior to death at 80 to 110 d. Animals were assessed as acyclic if they showed at least 8 consecutive d of cornified smears. The ovaries were removed (where possible on the day of oestrus) and were weighed and examined for the presence of recent (functional) corpora lutea and the oviducts were examined for the presence of eggs.

Table 1 shows the results of a low (30 µg) dose of TP combined with the maximum dose of each inhibitor compatible with survival to adulthood. This dose of TP did

not induce full masculinisation at 80 d of age; at this stage 20% of the animals still had functional corpora lutea and 30% showed irregular vaginal oestrous cycles. Nevertheless, the inhibitors OH-U and AMAN were capable of preventing this degree of masculinisation to a highly significant extent particularly in terms of ovarian weight ($P < 0.01$ and < 0.001 respectively). This was also true of a dose of AMAN one fifth of the maximum tolerated, although at this dose no clinical symptoms or disturbance of normal growth were observed during the neonatal period. The protein synthesis inhibitors (PURO and FU) were less effective but still provided a partial protection against this dose of TP ($P < 0.05$). Only ACT.D was completely without effect on the development of masculinisation in these animals. When these doses of inhibitors were given alone they had no significant effects on vaginal cycles, incidence of ovulation, ovarian morphology or ovarian weight.

The results of a similar experiment using 80 µg of TP are shown in Table 2. With this dose of androgen 90% sterility and 95% acyclicity were achieved and the ovarian weight was reduced to 40% that of controls. Again AMAN and OH-U produced effective protection against masculinisation ($P < 0.001$ in terms of ovarian weight); evidence of ovulation in the form of corpora lutea was present in 100% of rats given the higher dose of AMAN. The lower dose of AMAN still afforded clear protection against 80 µg of TP ($P < 0.01$); however, PURO failed to provide significant protection against this dose ($P > 0.10$). Once again ACT.D was completely without a protective effect.

In a further experiment a dose of 200 µg of TP was used and in this case 100% sterility and acyclicity were achieved (Table 3). A high degree of protection was again obtained with OH-U, the effect being highly significant in terms of all the parameters tested ($P < 0.01$ and $P < 0.001$). Indeed the ovaries were almost equal in weight to the controls which received the inhibitor alone. AMAN was markedly less effective against this high dose of TP but 33% of the animals showed signs of ovulation ($P < 0.05$) and their ovaries were significantly heavier than the TP controls ($P < 0.01$) although much lighter than those of rats treated with the inhibitor alone ($P < 0.001$).

It is clear from these results that substances that block the synthesis of DNA or of nucleoplasmic RNA can protect the newborn female rat from the permanent neural organising effect of neonatal androgen. Inhibition of protein synthesis is less effective in this respect, and inhibition of ribosomal RNA synthesis alone (as produced by the low doses of actinomycin D we have used¹⁴) is completely ineffective. The former may be because we were unable to achieve a complete block to protein synthesis in the brain. Three hours after the dose of puromycin, inhibition

Table 1 Effect of 30 µg of TP Combined with Inhibitors of DNA, RNA and Protein Synthesis on Subsequent Vaginal Cycles and Ovarian Function

Treatment	Dose of inhibitor µg per 10 g b.w. per injection	Vaginal cycles		Ovaries at 80 to 90 d				Bilateral ovarian weight mg per 100 g $\pm$ s.e.m.
		No. acyclic/ total	%	No. with recent corpora/total	%	No. with ova in oviduct/total	%	
Control	—	0/14 ‡	0	14/14 ‡	100	13/14 ‡	93	42.3 $\pm$ 1.7 ¶
TP 30 µg	—	7/10	70	2/10	20	1/10	10	24.5 $\pm$ 3.2
TP+OH-U	8,000	2/7	29	6/7 †	86	3/7	43	38.3 $\pm$ 3.1
TP+AMAN	0.3	1/9 †	11	8/9 †	89	6/9 †	67	36.2 $\pm$ 2.6 §
TP+AMAN	1.5	0/6 †	0	6/6 †	100	4/6 †	67	38.6 $\pm$ 1.6 ¶
TP+ACT.D	0.6	12/17	71	5/17	29	4/17	25	27.8 $\pm$ 3.6
TP+PURO	300*	3/8	38	5/8	63	5/8 †	63	36.4 $\pm$ 3.9 §
TP+FU	80	3/10	30	7/10 †	70	6/10 †	60	36.8 $\pm$ 3.2 §

Inhibitors given at 0 and 6 h after TP to 4-d-old rats (*given at 0, 5, and 10 h after TP).

† $P < 0.05$.

‡ $P < 0.01$ against TP alone by Fisher exact probability test.

§ $P < 0.05$.

|| $P < 0.01$.

¶ $P < 0.001$ against TP alone by Mann-Whitney U test.

of protein synthesis (by ^{35}S -methionine incorporation) was only 28% in the hypothalamus compared with 77% in the liver. Thus the lack of a protective effect of puromycin in these animals is still compatible with an obligatory role of protein synthesis in sexual differentiation of the brain.

The dependence of sexual differentiation on DNA synthesis, suggested by our results with hydroxyurea, has not been previously demonstrated. The failure of Barnea and Lindner¹¹ to achieve protection with hydroxyurea seems to conflict with this finding but, as these authors comment, very little is known of the time course of action of TP and it is quite possible that the effect may be more delayed than hitherto supposed. If this were the case our two large systemic doses of inhibitor given subcutaneously might well produce a longer lasting effect on the brain than their smaller doses given intracranially. This dependence is, however, entirely compatible with what is known of the process of hormone-induced differentiation in other tissues, notably the mammary gland¹⁵. It seemed important, therefore, to establish that some DNA synthesis was in fact continuing in the hypothalamus of the 4-d-old rat. We used ^3H -thymidine autoradiography to study this and have obtained evidence of DNA synthesis in both neuronal and glial cell nuclei throughout the brain, including the hypothalamus, at this age¹⁶. This is in accord with recent biochemical evidence obtained from whole brain fractionated into neuronal and glial nuclei¹⁷. Figure 1 illustrates a field in the anterior hypothalamic area but similar levels of labelling were observed in the medial preoptic area and the ventromedial/paraventricular region, the range being from 0.9 to 1.3% of cells labelled after 3 h exposure to ^3H -thymidine. The effect of TP on the frequency of thymidine labelling in this part of the brain is being investigated.

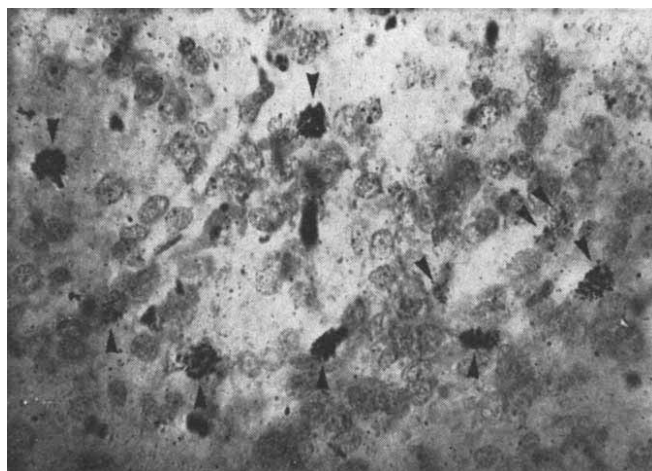


Fig. 1 Autoradiograph of parasagittal (8 μm) section from the anterior hypothalamic area (0.2 mm from the midline) of a 4-d-old female rat which received 50 μCi of ^3H -thymidine s.c. 3 h before death. Counter stained with nuclear fast red, exposed for 1 week at 4° C. Magnification $\times 390$. Ten labelled nuclei are visible (arrows).

The protective action of α amanitin is equally striking since it is highly effective at a dose of one fifth of the maximum tolerated by the neonatal rat and one twentieth of that used by Tata *et al.*¹³ to inhibit RNA synthesis *in vivo*. This suggests that the masculinisation process may be dependent on the synthesis of a few specific RNA molecules. It is possible that these may be histone or acidic nuclear-protein mRNA molecules the translation products of which would be necessary for the DNA synthesis that

Table 2 Effect of 80 μg of TP Combined with Inhibitors of DNA, RNA and Protein Synthesis on Subsequent Vaginal Cycles and Ovarian Function

Treatment	Dose of inhibitor μg per 10 g b.w. per injection	Vaginal cycles		Ovaries at 80 to 90 d		Bilateral ovarian weight	
		No. acyclic/ total	%	No. with recent corpora/total	%	No. with ova in oviduct/total	%
Control	—	0/14†	0	14/14†	100	13/14†	93
TP 80 μg	—	20/21	95	2/21	10	0/21	0
TP+OH-U	5,000	4/13‡	31	9/13‡	69	6/13‡	46
TP+AMAN	0.25	2/8‡	25	6/8‡	75	4/8‡	50
TP+AMAN	1.5	1/9‡	11	9/9‡	100	4/9‡	44
TP+ACT.D	0.6	12/13	92	2/13	15	1/13	8
TP+PURO	300*	4/6	67	2/6	33	2/6	33
							mg per 100 g $\pm$ s.e.m.
							42.3 $\pm$ 1.7
							16.8 $\pm$ 1.1
							32.7 $\pm$ 2.6
							29.4 $\pm$ 3.4§
							35.5 $\pm$ 2.6
							19.1 $\pm$ 1.9
							22.2 $\pm$ 3.1

Inhibitors given at 0 and 6 h after TP to 4-d-old rats (*given at 0, 5 and 10 h after TP).

† $P < 0.05$.

‡ $P < 0.01$ against TP alone by Fisher exact probability test.

§ $P < 0.01$.

|| $P < 0.001$ against TP alone by Mann-Whitney U test.

Table 3 Effect of 200 μg of TP Combined with Inhibitors of DNA and RNA Synthesis on Subsequent Vaginal Cycles and Ovarian Function

Treatment	Dose of inhibitor μg per 10 g b.w. per injection	Vaginal cycles		Ovaries at 95 to 110 d		Bilateral ovarian weight	
		No. acyclic/ total	%	No. with recent corpora/total	%	No. with ova in oviduct/total	%
Control	—	1/16†	6	15/16†	94	13/16†	81
TP 200 μg	—	13/13	100	0/13	0	0/13	0
TP+OH-U	4,000	2/11†	18	8/11†	73	6/11†	55
TP+AMAN	1.5	11/15	73	5/15*	33	3/15	20
OH-U control	4,000	0/8†	0	8/8†	100	6/8†	75
AMAN control	1.5	0/13†	0	13/13†	100	11/13†	85
							mg per 100 g $\pm$ s.e.m.
							33.6 $\pm$ 1.5
							16.1 $\pm$ 1.5
							35.0 $\pm$ 2.4
							23.8 $\pm$ 2.0‡
							36.6 $\pm$ 1.9
							35.4 $\pm$ 1.2

Inhibitors given at 0 and 6 h after TP to 4-d-old female rats.

* $P < 0.05$.

† $P < 0.01$ against TP alone by Fisher's exact probability test.

‡ $P < 0.01$.

|| $P < 0.001$ against TP alone by Mann-Whitney U test.

might follow. Hydroxyurea is known to block the synthesis of these specific mRNAs selectively¹⁸.

Although the precise mechanism of sexual differentiation remains obscure, it is apparent from these results that DNA, RNA and probably also protein synthesis is involved at an early stage; in this respect the process is closely analogous to hormone-induced differentiation in other tissues of the body, for example, in the mammary gland¹⁵ or the erythropoietic system¹⁹.

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Dissociation of Stimulatory and Synthetic Phases in the Induction of Tyrosine Hydroxylase

RESERPINE treatment causes an increase in the activity of tyrosine hydroxylase (T-OH) in the superior cervical ganglion of the rat. A single systemic injection of the drug produces a rise in enzyme activity that reaches a maximum 48–72 h later¹. No change in T-OH is seen if the preganglionic cholinergic tract is cut at the time of reserpine administration or if animals are pretreated with the ganglion blocking agents pempidine or chlorisondamine^{2,3}. In the cat, direct electrical recording from the preganglionic cervical sympathetic trunk has revealed increased neuronal firing after reserpine administration⁴. These findings suggest that the rise in T-OH activity elicited by reserpine is mediated trans-synaptically through an increase in ganglionic transmission.

Little is known about the sequence of events that occurs between the injection of reserpine and the increase in T-OH activity. We have used a combination of *in vivo* administration of reserpine with subsequent *in vitro* maintenance of superior cervical ganglia to achieve a temporal dissocia-

tion of the trans-synaptic stimulation of the ganglia from the actual increase in T-OH activity. We have also used this system to assess the importance of continuing protein synthesis in the ganglion for the development of this increase in enzyme activity.

Adult male Wistar rats (250–350 g) were injected subcutaneously with 5 mg kg⁻¹ reserpine (British Drug Houses, Poole) in 20% ascorbic acid or with the vehicle alone. Superior cervical ganglia were removed 24 or 72 h later and the ganglion sheaths removed. The ganglia were then either frozen at -20° C or placed in organ culture. The organ culture system involved placing ganglia on Millipore filters supported on metal grids in Falcon organ culture dishes (Becton, Dickinson and Co., Wembley, Middlesex). Eagle's minimum essential medium was supplemented with glucose (to 600 mg%), neonatal calf serum (10% v/v) and penicillin and streptomycin (100 IU ml⁻¹). The dishes were incubated at 37° C in an incubator perfused with humidified 95% O₂ and 5% CO₂ for 48 h. After incubation, ganglia were washed thoroughly in basic salt solution and stored at -20° C until assayed. Individual ganglia were homogenised in 20 or 60 µl of 5 mM Tris buffer, pH 7.4, containing 0.1% Triton X-100. Tyrosine hydroxylase activity was assayed in 10 µl aliquots using L-tyrosine (2,3,³H-side chain) as substrate at a concentration of 8 µM (ref. 5). Protein content was determined using the method of Lowry⁶.

Table 1 Tyrosine Hydroxylase Activity in Rat Superior Cervical Ganglia Following Reserpine Treatment

	T-OH activity as % <i>in vivo</i> controls
<i>In vivo</i> controls	100 ± 11
Reserpine 24 h	102 ± 6
Reserpine 72 h	186 ± 10
Culture controls	86 ± 4
Reserpine culture	158 ± 9
	(185 ± 10% of culture control)

The tyrosine hydroxylase activity in the five experimental groups is expressed as a percentage of the *in vivo* controls. Means and s.e.m. are based on groups of thirty ganglia (pooled results from five experiments). The tyrosine hydroxylase activity in the *in vivo* controls was 350 pmol dopa per h per mg protein. 'Reserpine 24 h' and 'reserpine 72 h' refer to values obtained from ganglia removed from animals 24 h and 72 h after administration of reserpine (5 mg kg⁻¹, subcutaneously). 'Culture' results were from ganglia maintained in organ culture for 48 h; 'Reserpine culture' results from ganglia explanted 24 h after administration of reserpine.

Five experimental groups were studied: '*in vivo* controls' (ganglia from rats injected with vehicle alone 24 or 72 h previously), 'reserpine 24 h' (ganglia from rats injected 24 h previously with reserpine), 'reserpine 72 h' (ganglia from rats injected 72 h previously with reserpine), 'culture controls' (ganglia excised from rats injected with vehicle alone 24 h previously and subsequently maintained for 48 h in organ culture), 'reserpine culture' (ganglia from rats injected 24 h previously with reserpine and subsequently maintained for 48 h in organ culture). The *in vivo* control level of T-OH activity was 350 pmol dopa per h per mg protein. No change in T-OH activity was observed in the reserpine 24 h group when compared with the *in vivo* controls. The reserpine 72 h ganglia, however, showed an 86% increase (Table 1). Over the 48 h culture period, the T-OH activity in the culture control ganglia fell on average to 86% of the *in vivo* controls. On the other hand, the reserpine culture group showed a 58% rise in T-OH activity relative to *in vivo* control and an 85% rise relative to 'culture control'. Thus ganglia removed from the animal before any increase in T-OH activity had developed showed an increase in the enzyme in culture comparable with that which would have been achieved *in vivo*. These findings

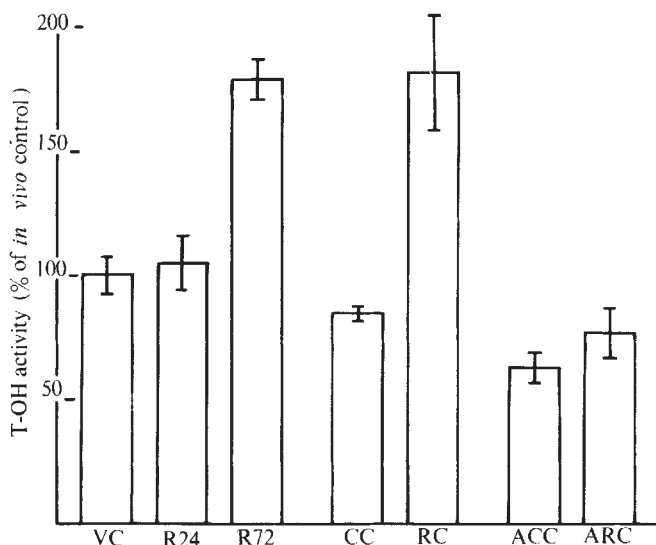


Fig. 1 Effect of anisomycin on the increase in tyrosine hydroxylase activity following reserpine treatment. The means $\pm$ s.e.m. are expressed as percentages of *in vivo* control values. Six ganglia were assayed in each group. The groups were: VC=*in vivo* controls, R24=reserpine 24 h, R72=reserpine 72 h, CC=culture control, RC=reserpine culture, ACC=anisomycin ($2 \mu\text{g ml}^{-1}$) culture control, ARC=anisomycin ($2 \mu\text{g ml}^{-1}$) reserpine culture. Mean tyrosine hydroxylase activity of *in vivo* control group was $350 \text{ pmol dopa h}^{-1} \text{ per mg protein}$. For details see Table 1 and text.

suggest that any stimulation of the ganglion in the intact animal beyond that achieved within 24 h of a single injection of reserpine is unimportant to the expression of increased T-OH activity.

To investigate the role of protein synthesis in the development of increased T-OH activity one group of reserpine culture ganglia was maintained for 48 h in the presence of anisomycin, a specific blocker of peptide bond formation⁷, at a concentration of $2 \mu\text{g ml}^{-1}$ in the culture medium. The enzyme activity was compared with that of a similar group of reserpine culture ganglia maintained for 48 h in normal medium. The T-OH rise normally seen after reserpine was completely abolished in the anisomycin-treated group (Fig. 1). This finding that inhibition of protein synthesis in the isolated ganglion abolished the reserpine-stimulated rise in T-OH activity confirms earlier interpretations of the effects of cycloheximide administered to the whole animal⁸. Our results clearly demonstrate that an important site of this effect of protein synthesis inhibitors is the ganglion itself.

By removing the ganglion from the animal and maintaining it in culture we have been able to show that after a period of stimulation the adrenergic cell bodies can produce an increase in T-OH activity when isolated from the neural and humoral changes produced *in vivo* by reserpine. Furthermore, this increase in enzyme activity is dependent on protein synthesis.

A recent study using actinomycin D suggests that the transcriptional phase of the rise in T-OH activity of rat stellate ganglia evoked by a 2 h exposure to stress is complete 24 h later⁹. Other workers have found that 12 h after insulin treatment actinomycin D can no longer prevent the insulin-induced rise in adrenal T-OH activity¹⁰. The time course of events following reserpine treatment has still to be elucidated.

The system we have described should provide a useful means of investigating this and other questions concerning the cellular mechanisms by which neurones respond to trans-synaptic stimulation.

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Preparation of Frozen Hydrated Tissue Sections for X-ray Microanalysis in the Scanning Electron Microscope

THE technique of X-ray microanalysis, using a finely focused beam of electrons to excite characteristic X rays from the elements present in a specimen, is proving to be a powerful tool in the investigation of biological systems. Its main limitation has been that, in common with all electron-optical methods, the specimen must first be stabilised by removal or immobilisation of cell and interstitial water. Conventional chemical fixation techniques give excellent structural preservation, but also cause gross permeability changes in cell membranes and, together with subsequent dehydration, lead to substantial loss or redistribution of free electrolytes. Freeze drying suffers from a similar limitation, in that removal of water must inevitably redistribute electrolytes which move to the remaining structures such as cellular membranes.

To study the distribution of water-soluble ions in biological systems, we have therefore developed a technique for immobilisation of water and solutes by rapid freezing, followed by sectioning and examination of the tissue while still in the frozen, hydrated state, that is with cellular and interstitial water retained as ice at a low temperature. A similar approach is also being adopted by Gehring *et al.*¹ and by Hutchinson (private communication).

Full details of the preparative procedure are given elsewhere in papers on technique²⁻⁵: the principal purpose of this communication is to describe our first successful results. The specimens were salivary glands isolated from 2 to 3-d-old larvae of the blowfly (*Calliphora*). These were mounted in either insect blood or a balanced medium⁶ on an aluminium chuck and quench frozen in liquid fluorocarbon at -150°C (ref. 3). The chuck was transferred to a Slee retracting microtome in a large cryostat chamber at -85°C , and sections 1 to $2 \mu\text{m}$ thick were cut using a steel knife. Sections were picked up on aluminised nylon support films⁵, stretched over aluminium collars designed to fit into a copper specimen holder for examination and microanalysis. The collars with

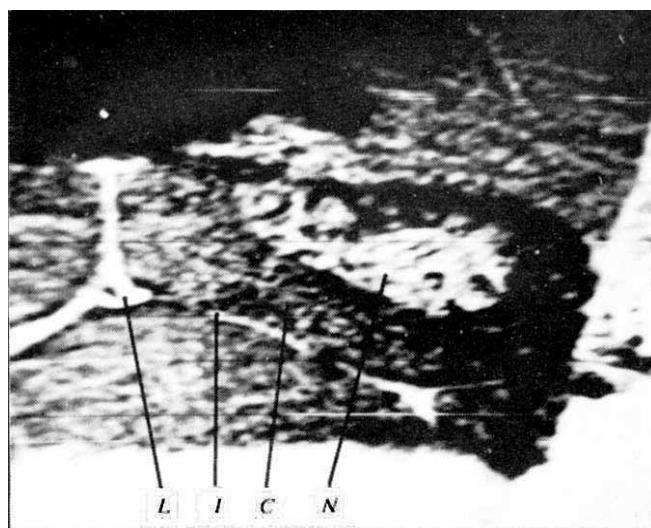


Fig. 1 Scanning transmission electron micrograph showing a frozen, hydrated transverse section of a blowfly larval salivary gland. C, Cytoplasm; N, nucleus; L, lumen; I, intercellular cleft.

sections attached were immediately immersed in liquid nitrogen, where they could be stored before fitting into the specimen-holder. Contamination of microtome and sections by surface ice was prevented by careful exclusion of atmospheric air during sectioning and transfer: the cryostat chamber was divided by a vertical polyethylene curtain separating the manipulation area from the microtome, and all manipulations were carried out either in the cryostat or under liquid nitrogen.

For critical work using diffracting spectrometers and high probe currents, it would be desirable to coat the mounted specimens, still maintained at a low temperature, with a further layer of evaporated metal to improve their stability and conductivity. In the experiments described here, where an energy-selective spectrometer and moderate beam currents were used, this complication was found not to be necessary and was omitted. The mounted sections, under liquid nitrogen, were enclosed in a protective metal cap⁵ and transferred directly to the pre-cooled stage of a Stereoscan S4 scanning electron microscope (Cambridge Scientific Instruments Ltd).

The cold-stage consists of a standard Cambridge Instruments Series 200 microanalysis stage with hot-cold module, which we have modified to allow the examination of thin specimens in the transmission mode³. The detector for transmitted electrons is a silicon solar-cell (Centralab, California) approximately 2 mm in diameter, mounted about 40 mm below the specimen and coupled through a pre-amplifier to the video system of the Stereoscan. Our modifications have also increased the working distance from the specimen to the final lens of the microscope to about 18 mm. This allows us to insert the 'nose' of a lithium-drifted silicon X-ray detector (active area 25 mm²; Quartz et Silice, Paris) close to the horizontally mounted specimen.

Figure 1 shows the scanning transmission electron image of a transverse section of a salivary gland, taken with an operating potential of 30 kV. Resolution at this potential is severely limited by scattering of electrons in the specimen, which is relatively thick compared with those normally used for transmission electron microscopy. Contrast is good, however, and the major cellular features are clearly identifiable.

X-ray microanalysis was carried out by advancing the detector as close as possible to the specimen (distance 10 to 15 mm) and allowing the electron beam to scan an area of

specimen 5 μm square. Figure 2 shows X-ray spectra from areas in the nucleus, cytoplasm and lumen of the gland, each obtained with a 30 kV, 4 nA probe over an irradiation period of 100 s. The stage temperature was -160° C. The spectra show clearly the presence of a large concentration of potassium in the cell, but not in the lumen. Phosphorus and sulphur are also present in the cell; signals from copper, iron and silicon are background resulting from scattered electrons, and that from aluminium is generated mainly in the supporting film. Although these spectra do not by themselves permit accurate quantitative microanalysis, they are sufficient to show that ions are retained in the specimen during preparation. By using stable inorganic standards and the higher X-ray resolution obtainable with diffracting spectrometers, fully quantitative measurements will be possible.

Stability of the specimen under the beam was investigated by using the X-ray continuum intensity as a measure of the total mass in the irradiated portion of the specimen⁷. In Fig. 3, A shows local mass as a function of time in conditions similar to those used for microanalysis. The curve is similar to those obtained by Hall and Gupta⁸ from cooled, freeze-dried tissue sections, showing only a very slow loss of material due to chemical and thermal damage by the high-energy electron beam. The early loss of mass which is normally found with room-temperature specimens was found in the present experiments only after the specimen had been warmed (Fig. 3B). It is thus very unlikely that any substantial loss or redistribution of inorganic ions could take place as a result of irradiation.

Apart from a brief period at about -80° C during sectioning, the temperature of the specimen during preparation and transfer never rose above -120° C, and it was always protected from exposure to the air. It is therefore most unlikely that any melting or drying would occur. Direct evidence that the specimen as examined was indeed hydrated

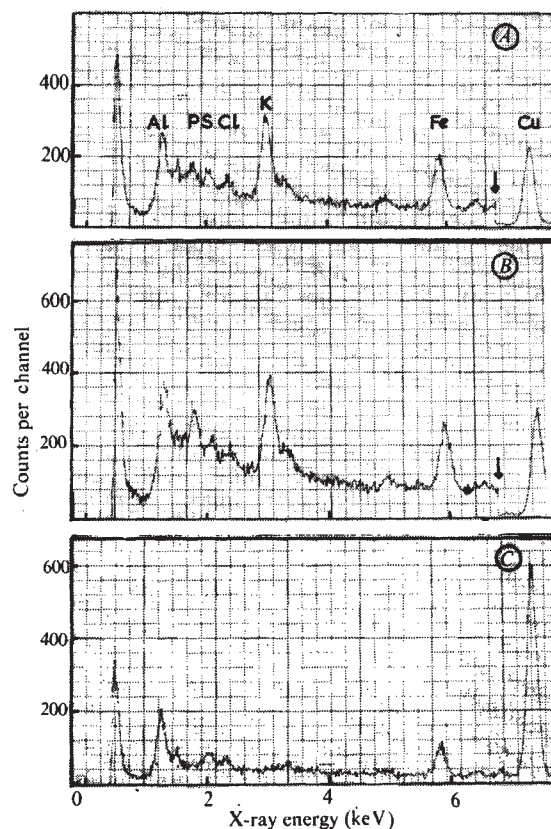


Fig. 2 X-ray spectra from the specimen of Fig. 1. A, Nucleus; B, cytoplasm; C, lumen. In A and B the vertical scale was reduced by a factor of 5 at the arrowed point, to keep the copper peak on scale.

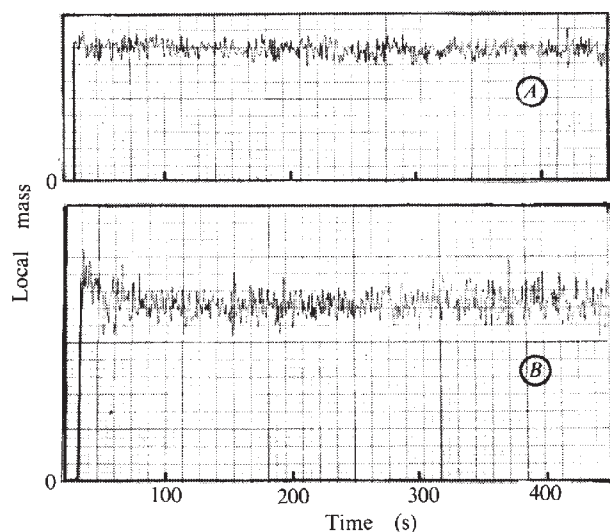


Fig. 3 Local mass (arbitrary units) against time during irradiation of the specimen. A, At -160°C ; B, at room temperature, after sublimation of ice from the specimen.

was obtained by allowing the stage temperature to rise slowly, while continuing to watch the scanning transmission and secondary-electron 'reflection' images. At about -75°C , a transient rise in vacuum pressure indicated the sublimation of ice from stage and specimen, and was accompanied by an increase in contrast of the transmission image. The secondary-electron image, which had previously been almost featureless, now showed recognisable outlines of the cells (this is consistent with the results of Gehring *et al.*¹, who found it necessary to etch the surface of a frozen bulk specimen, in order to obtain a useful secondary-electron image).

We believe that Fig. 1 is the first scanning transmission electron image to be obtained from a frozen, hydrated tissue section, with firm evidence that the specimen did not suffer dehydration during preparation, transfer or examination. Although these results are of a preliminary nature, and do not in themselves significantly advance our knowledge of cells and tissues, we believe that they demonstrate the feasibility of a technique which is capable of providing answers to numerous biological problems. From the present results to the analysis of ion distributions as they exist in living tissues, on a scale of $1\text{ }\mu\text{m}$ or less, is a step involving no more than the use of more specialised instrumentation already commercially available.

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Number of Ouabain-binding Sites in Fibroblasts from Normal Subjects and Patients with Cystic Fibrosis

ONE of the characteristics of cystic fibrosis (CF), an autosomal recessive disease, is a defect in the net sodium transport in the eccrine glands resulting in an increased concentration of sodium in the sweat¹. This defect has yet to be characterised as an extrinsic and/or intrinsic phenomenon. Mangos and McSherry² demonstrated an unidentified factor in the sweat from CF patients which inhibited sodium reabsorption in the rat parotid duct after retrograde perfusion. The relationship of the sweat factor to factors observed in saliva³ and sera^{4,5} of CF patients has yet to be elucidated.

Although there is no direct evidence for an intrinsic defect within the gland, studies of erythrocytes indicate that a defect could exist. Balfe *et al.*⁶ reported abnormal sodium transport in erythrocytes from CF patients; Lapey and Gardner⁷ demonstrated a decrease in the pump II component of sodium efflux in male CF patients; and Horton *et al.*⁸ reported a decrease in calcium-activated ATPase in CF erythrocytes. Fitzpatrick *et al.*⁹ observed an altered electrophoretic pattern of CF erythrocyte membranes, which they interpreted as an altered calcium-binding component in the membrane. However, Hadden *et al.*¹⁰ could not demonstrate significant differences in the sodium-potassium ion exchange and in the calcium efflux pump of CF and normal erythrocytes when comparisons were matched for age and sex.

Recently, binding of ^3H -ouabain to the cell membrane has been used to determine the number of ouabain-binding sites per cell in red blood cells¹¹⁻¹³, in cultured Girardi heart cells¹⁴, and in HeLa cells^{11,15,16}. These studies demonstrated a direct correlation between the number of ouabain-binding sites per cell (at low ouabain concentrations) and the Na^+-K^+ ATPase activity.

As Hadden *et al.*¹⁰ have said, the differences observed between CF and normal erythrocytes are difficult to interpret because the CF cells may be affected by the anoxia, infection, malabsorption, liver disease and vitamin or antibiotic therapy seen in CF patients. Recently, Fletcher and Lin¹⁸ found no differences in the Na^+-K^+ ATPase activity, measured in a heavy particulate fraction from fibroblasts of normal subjects and CF patients after cell homogenisation. Because of possible complications in the erythrocytes from CF patients, and since the activity observed in a cell homogenate may not express the situation in the intact cell, we have looked for differences in the binding of ^3H -ouabain in cultured normal and CF human diploid fibroblasts.

Human fibroblast cell strains were established from skin biopsy explants. The fibroblasts, after outgrowth, were frozen and stored in liquid nitrogen. The three normal and three CF fibroblast cell strains used had undergone less than ten subcultures since the establishment of the cell strain from the biopsy. The fibroblasts were cultured in F-12 (Gibco) containing 15% foetal calf serum (Gibco) and 100 U ml^{-1} of penicillin and $100\text{ }\mu\text{g ml}^{-1}$ streptomycin.

The number of binding sites for CF and normal human diploid fibroblasts was determined on cells in log phase (Fig. 1). The initial determination was made 24 h after inoculation of

the cultures and additional determinations were carried out on days 2 and 4. As Figure 2 shows, we observed no appreciable differences in the number of ouabain-binding sites between normal and CF cell strains. The mean number of sites per cell for the normal fibroblasts was $7.0 \times 10^6 \pm 2.3$ s.d. and for the CF fibroblasts $8.3 \times 10^6 \pm 1.5$ s.d.

Boardman *et al.*¹⁶ found that HeLa cells, after exposure to sublethal levels of ouabain for 24 h, had an increased number of ouabain-binding sites per cell and a concomitant increase in their Na^+/K^+ ATPase activity. Thus the cells compensated for the decreased Na^+/K^+ ATPase activity caused by ouabain inhibition of the enzyme. Since there was no apparent change in the number of binding sites during logarithmic growth of the CF cell strains (Fig. 2), it seems that the ciliary dyskinetic factor which accumulates in the media of CF fibroblasts¹⁷ does not interfere with their Na^+/K^+ ATPase activity. Furthermore, the constancy in the number of ouabain-binding sites in CF fibroblasts indicates that the factor does not appreciably affect the permeability of the plasma membrane; that is, an increased number of binding sites would be expected¹⁶ to maintain the normal intracellular ionic environment if the permeability of the membrane had increased.

Our observations also agree with the studies of Fletcher and Lin¹⁸, who observed no differences in the membrane ATPase activity and its response to inhibition by ouabain and ethacrynic acid in CF and normal fibroblasts.

We were also unable to observe any differences in the kinetics of ouabain-binding between normal and CF fibroblasts in K^+ -free phosphate-buffered saline solution, and the results were essentially the same as those seen in HeLa cells^{15,16}; that is, saturation was achieved within 30 min at 37°C (2.0×10^{-7} mol l^{-1} ouabain) and remained constant for more than 1 h.

The studies on the Na^+/K^+ ATPase in CF fibroblasts, measured in a heavy particulate fraction from a cell homogenate, have indicated no observable differences in the specific activity of the Na^+/K^+ ATPase nor any alteration in the inhibitory pattern of the Na^+/K^+ ATPase *per se*¹⁸. Using intact cells, we observed no alterations in the kinetics of binding of ouabain nor any alteration in the total number of binding sites. These data indicate that the Na^+/K^+ ATPase functions normally in the CF fibroblast.

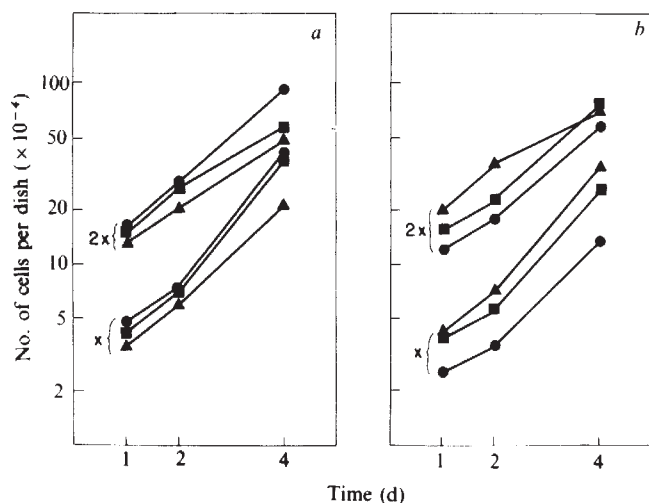


Fig. 1 The cell strains were grown to confluency and trypsinised. The resultant suspensions of cells were inoculated into P-60 Falcon tissue culture dishes at an approximate cell concentration of 4 (X) and 8 ($2X$) $\times 10^4$ cells per dish. The cells were cultured in F-12 (Gibco) containing 15% foetal calf serum. At day 1, 2, and 4, 3 P-60 Falcon plastic dishes for each cell strain at each cell density were washed with saline, trypsinised and the total cell number per dish was determined. Results shown are the mean of the three determinations. *a*, Normal fibroblast: strains were (▲), No. 17; (■), No. 18; (●), No. 31. *b*, CF fibroblast: (▲), No. 2; (■), No. 4; (●), No. 24.

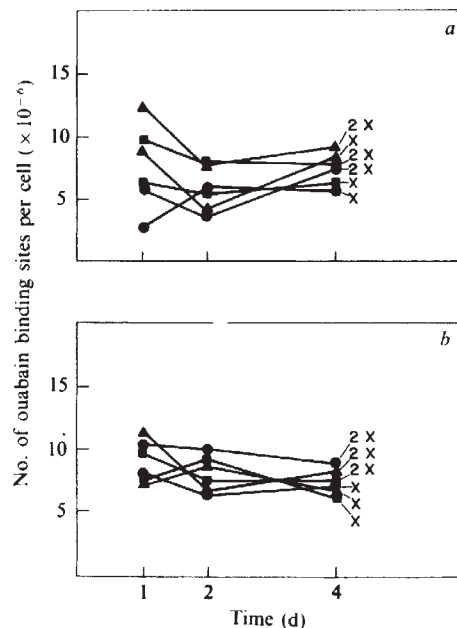


Fig. 2 The cell culture media were removed and the cells were washed with a potassium-free phosphate-buffered saline, pH 7.4, 37°C , which contained 0.01% glucose (PBS-g). The ^3H -ouabain (New England Nuclear Corp.) was added in a PBS-g solution (37°C), and the cells were incubated for 30 min. The ^3H -ouabain solution was removed, and the cells were washed four times with excess quantities of PBS-g at 0°C . The final wash was removed, the cells were trypsinised, and the entire contents of the culture dish were transferred to counting vials for liquid scintillation counting. The ouabain concentration used was 2.08×10^{-7} mol l^{-1} , and the specific activity was 2.3×10^7 mol per d.p.m. Each point corresponds to the mean of three determinations. X and $2X$ correspond to the inoculum cell concentration. The cell number per dish at the time of each determination is seen in Fig. 1. *a*, Normal fibroblast: strains were (▲), No. 17; (■), No. 18; (●), No. 31. *b*, CF fibroblast: (▲), No. 2; (■), No. 4; (●), No. 24.

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Resistance to Marek's Disease in Immunologically Deficient Chickens

A CELL-ASSOCIATED herpesvirus has been shown to induce Marek's disease (MD), a naturally occurring malignant neoplasm of chickens. The mechanism of tumorigenesis or the factors that mediate natural or acquired resistance to MD are not well understood. Information on these aspects is needed for a better understanding of the basic mechanisms of this and possibly other herpesvirus-induced neoplasms.

Inherited resistance of chickens to MD has been known for many years¹, and several inbred lines selected for natural resistance are available. We observed that chickens of one such line, designated line 6, when exposed to MD virus (MDV), developed persistent viraemia and antibody but no clinical disease². The mechanism of resistance is unknown, but in confirmation of Calnek's observations on another genetically resistant line³, we found that line 6 chickens produced a better virus neutralising (VN) antibody response than simultaneously inoculated genetically susceptible chickens. Thus, it was postulated that genetic resistance to this disease has an immunological basis and may depend on the ability of chickens to produce VN antibody. In testing this postulate I reasoned that since antibody synthesis is a function of cells derived from the bursa of Fabricius⁴, response to MDV inoculation of bursectomised genetically resistant chickens should provide proof of the involvement of humoral antibody in this type of resistance.

Two experiments were conducted. The White Leghorn chickens used had maternal MD antibody at hatching. Chickens of line 6 known to be resistant to clinical MD^{2,5} were either surgically bursectomised on the seventeenth day of embryonic life or sham operated as described by Van Alten *et al.*⁶. Surgical bursectomy at or before this stage eliminates or severely impairs the ability of chickens to synthesise both IgM and IgG⁷. One week after hatching, bursectomised, sham operated and untreated chickens of line 6 and line 7 hatchmates (highly susceptible line) were either inoculated with the JM 19 clone⁸ of MDV (1.8×10^5 and 1.0×10^5 PFU per chicken in experiments 1 and 2 respectively), or left as uninoculated controls. In both experiments 33–44% of hatched chickens from bursectomised and sham operated groups died of herniated gut before virus inoculation. Each group was maintained in a separate Horsfall-Bauer isolator under negative pressure and was given regular feed and medicated water (Floxaid, 2 ounces per 50 gallons of drinking water, Merck). At 4 and 6 weeks old, each bursectomised and sham operated chicken was inoculated intramuscularly with 10^9 sheep erythrocytes suspended in 0.5 ml of phosphate-buffered saline. The inoculum was deposited at either a single site or three different sites in the same bird.

In both experiments, 8 weeks after inoculation, all surviving chickens were bled by cardiac puncture and then killed. Certain groups were tested for levels of viraemia by inoculating white blood cells from individual chickens onto secondary cultures of duck embryo fibroblasts⁹ and counting foci of

cytopathic effects 10–14 d later. Plasma or serum samples were tested by some or all of the following tests: agar gel precipitin (AGP)¹⁰, immunofluorescent (IF)¹¹ and VN tests for anti-MDV antibody²; haemagglutination (HA) test for anti-sheep erythrocyte agglutinins¹²; radial diffusion test^{13–15} for values of IgM and IgG (conducted by Dr M. D. Cooper). All chickens were examined grossly and the following sections were taken for histological examination: both vagi, right and left sciatic, and right and left brachial plexuses, gonads, spleen and bursa. If bursal remnants could not be detected grossly, a small area at the usual location of the bursa was sectioned. All sections were stained with haematoxylin and eosin and additional sections of spleen were stained with methyl green-pyronin also¹⁶.

Since the design and results of both experiments were similar, data were pooled and are given in Tables 1 and 2. Of sixteen survivors in the bursectomised group, eleven had no detectable bursal remnant. These eleven were all deficient in bursa functions, that is, they produced no anti-sheep erythrocyte agglutinins, they had no detectable germinal centres in spleen and their sera lacked detectable levels of IgM and IgG (Table 1). The five chickens with bursal remnants and all sham operated chickens had well developed bursa-dependent functions (Table 1). The response of bursectomised, sham operated and untreated line 6 chickens to MDV is given in Table 2. Only the chickens that lacked gross and microscopic evidence of bursa and bursa-dependent functions are included in the bursectomised group. The incidence of MD in genetically susceptible line 7 chickens was high (approximately 90%), indicating that MDV inoculum was highly pathogenic. However, clinical MD did not develop in bursectomised, sham operated and untreated chickens of genetically resistant line 6. Bursectomised chickens lacked AGP, IF and VN antibody against MDV in contrast to the high incidence of antibody detectable by all three tests in sham operated and untreated groups. Four of eleven bursa-deficient chickens had viraemia, evidence that chickens in this group were infected with MDV. In one experiment, five of thirteen uninoculated control chickens of line 6 had AGP antibody indicating inadvertent exposure to MDV. However, infection in this control group apparently did not affect the outcome of this study because in the second experiment where control chickens remained free of MD the results were similar.

In this study, although a limited number of bursectomised chickens were available, the results were considered valid because a complete morphological and functional depletion of bursa was achieved without endangering the functions of other tissues or systems. Also, since line 6 chickens are highly resistant to MD, any variation in their response would have been detectable even among the small numbers used.

These experiments demonstrated that bursectomised chickens of line 6, although devoid of immunoglobulins, maintained their resistance to MD. Unpublished studies of Payne and Rennie (quoted by Payne¹⁷) reportedly produced similar results. Although these studies strongly suggest that the ability to synthesise humoral antibody was not a critical factor

Table 1 Bursa-dependent Functions in Bursectomised and Sham Operated Line 6 Chickens

Embryonic treatment	Bursal remnant*	No. of chickens	Germinal centres per spleen section†		HA titre (log ₂) ‡		mg% IgM in serum		mg % IgG in serum	
			Mean	Range	Mean	Range	Mean	Range	Mean	Range
Bursectomy	Absent	11	0.0	—	0.0	—	0.0	—	0.0	—
Bursectomy	Present	5	6.15	2–11	6.8	3–≥9	29.4	13.5–44.7	48.9	26.0–95
Sham operated	Present	18	15.45	3–36	7.2	5–≥9	27.6	13.5–55.1	109.3	0.0–201.0

Data were pooled from two experiments.

* Detectable by gross and (or) microscopic examination.

† Spleen was sectioned approximately through the middle and all germinal centres present in the entire cross section were counted.

‡ Sheep erythrocyte agglutinins.

Table 2 Response of Bursectomised, Sham Operated and Untreated Chickens of Line 6 to Marek's Disease Virus (MDV) Inoculation

No. of chickens	Embryonal treatment	Inoculum	Viraemia		AGP +/ tested	MD-Antibody†		Mean titre	No. died	MD No. with lesions‡ at termination	Total MD
			+/ tested	Mean* titre		VN +/ tested	IF +/ tested				
11	Bursectomised	MDV§	4/11	2.6	0/11	0/11	0/11	0	0	0	0/11
18	Sham operated	MDV	2/18	0.25	12/18	18/18	18/18	≥ 142	0	0	0/18
29	None	MDV	NT	NT	28/28	NT	28/28	≥ 134	0	0	0/28
28	None	None	NT	NT	5/28¶	NT	NT	NT	0	0	0/28

Data are pooled from two experiments. NT, not tested.

* PFU per 10⁷ white blood cells.

† AGP test was done on undiluted sera. For VN test, all samples were diluted 1 : 20. The IF antibody titre corresponds to the reciprocal of highest serum dilution giving a positive reaction (1 : 160 was the highest dilution tested).

‡ Gross or microscopic.

§ The MDV inoculum induced MD in approximately 90% of simultaneously inoculated line 7 chickens.

¶ Presence of AGP antibody indicates inadvertent exposure to MDV of this group in one experiment.

in resistance to MD, the following points must be considered before definite conclusions are reached.

(1) It can be postulated that bursa-derived (B) cells act as target cells for lymphoma formation by MDV and that embryonic bursectomy eliminated all available B cells from the host. Indeed, in lymphoid leukaemia, another haematopoietic neoplasm of chickens, neoplastic transformation originates in the cells of the bursa of Fabricius and bursectomy during early stages of infection prevents clinical disease¹⁸⁻²⁰. Thus, by this reasoning, lack of MD in bursectomised chickens was due basically to the lack of target cells. Although conclusive proof is lacking, available data suggest that the target cell in MD is not a B cell. The most direct evidence comes from the observation that MD lymphomas can be induced in susceptible, bursectomised, X-irradiated, agammaglobulinaemic chickens²¹. However, in this study, bursectomy and X-irradiation were performed at hatching, and since bursa-derived cells peripheralise during embryonic stages, it is possible but unlikely²² that some B cells were available at the time of virus inoculation. Conclusions of Foster and Moll that bursectomy decreased MD²³ were not confirmed by others who found bursectomy either to have no effect^{21,24} or to enhance the severity of MD in susceptible chickens^{25,26}. Also, Hudson and Payne²⁷ and Rouse *et al.*²⁸ found that in MD lymphoma, most lymphoid cells (80% or more) were of thymic origin.

(2) Line 6 chickens used in this study had maternal antibody against MDV at hatching and bursectomy presumably did not influence the protective effect this antibody may have had on their response. Although this argument cannot be settled until after a similar study of line 6 chickens without maternal antibody, it seems unlikely that maternal antibody alone was responsible for the resistance in these chickens. Studies where the protective effect of maternal antibody was quantitated²⁹ showed that susceptible chickens with maternal antibody could neutralise approximately 1 log₁₀ of cell-associated virus. Since in my study, line 6 chickens were challenged with 10⁵ or more PFU of pathogenic MDV, and a dose of 1 PFU or less can induce neoplastic response in the susceptible chicken⁵, it seems unlikely that maternal antibody seriously affected the outcome of this study. Nonetheless, in view of Calnek's observation³⁰ that maternal antibody was important in the expression of early resistance of N line chickens, the role of maternal antibody in MD needs further clarification.

The mechanism of resistance to MD is not clear. Suggestions include (a) selective ability of resistant chickens to produce VN antibodies^{2,3}; (b) cell-mediated immunity¹⁷; (c) increased production in resistant chickens of non-specific inhibitors such as interferon³¹. Although these possibilities may not be mutually exclusive, the study reported here clearly demonstrates that ability to produce antibody is not required in the expression of resistance to MD.

I thank Dr M. D. Cooper, University of Alabama, for demonstrating embryonic bursectomy and for conducting tests

for IgG and IgM. I also thank Drs H. G. Purchase and R. L. Witter for advice and assistance. Chickens were made available through Mr H. A. Stone. Mrs Evelyn Young and Mrs Karen Juntikka provided technical assistance.

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book reviews

Nutrition reviewed

World Review of Nutrition and Dietetics. Edited by Geoffrey H. Bourne. Vol. 17 pp. xii+318; vol. 18 pp. 336. (Karger: Basel, London and New York, September and October 1973.) Vol. 17: Sfr. 144; £20.90; \$57.75; vol. 18: Sfr. 165; £23.95; \$59.15.

THE two latest volumes of this ambitiously named series contain three quite different types of contributions; one group is composed of technical reports of particular aspects of biochemical research; the second, consisting of two papers, contains accounts of the changes in food supply and their consequent effect on nutrition in different communities; while the third, again made up of two contrasting pieces of writing, deals with the very different philosophical outlooks with which workers of different origins have come, after a generation of hard experience, to view the practice of applying nutrition and dietetics to the problems of diverse communities. In spite of the wealth of scholarship deployed in the 654 pages of the two books, it would be a naive purchaser who, after paying the combined price of £44.85, considered that he had received his money's worth.

Some of the research reviews are excellent. E. B. Olsen and H. F. De Luca contribute an admirable paper to volume 17 on the recent advances in understanding of the metabolism and mechanism of action of vitamin D. That by I. Szelenyi in the same volume on magnesium and its significance to cardiovascular and gastro-intestinal disorders is also stimulating, as a review article should be. For anyone who wants to know all about the use of molasses for feeding cattle or about the use of citrus fruits for feeding people (apparently more citrus fruit is eaten than any other kind), T. R. Preston has an exhaustive paper in volume 17 and J. Kefford an equally comprehensive one in volume 18. C. G. King contributes a good review covering the biological synthesis of ascorbic acid which possesses the added advantage of being succinct; the paper by J. T. Snook on protein digestion is equally good.

The two articles, one by S. C. Hsu describing what has happened to nutrition in Taiwan between 1945 and 1970, in volume 17, and the other by E. Ferber reviewing conditions in Yugoslavia between 1953 and 1963, in volume 18, take us into a different area of nutrition and dietetics. The minutiae of

the biochemistry of vitamins, of magnesium and water-borne goitrogens, about which E. Gaitan has an article in volume 17, are some of the essential bricks from which our knowledge of nutrition is built up. What use can be made of the edifice of scholarship is demonstrated in the narrative of communities of real people. It is easy to see that the work in Taiwan was influenced by American thinking. The tables of figures are all there, the dietary surveys, anthropometric data and plans to regulate the labelling of food and provide nutrition education for the public. In brief, the story is one of rapidly increasing prosperity, increased consumption of food in general and fish, pork and chicken in particular, bigger children, the disappearance of nutritional deficiency diseases and—you've guessed it—a rise in the mortality from vascular disease and an increased incidence of gout. Here, indeed, is the full gamut of nutritional science.

The narrative in Yugoslavia is the same but with a difference. Here too a rural population took to the industrial life. The people left the villages to work in the towns where imported food was provided. As they became richer they too ate better while those left behind had a poorer diet. But while this difference persisted and, as Ferber points out, despite the intrusion of combines, cars and television, regional dietary habits and customs remained. Nevertheless, in the country as a whole the consumption of meat, fruit and vegetables rose while the proportion of cereals fell. At the same time pellagra and rickets dwindled away. Ferber surely has the last word when he writes "all efforts of the health policy group would be futile without the advancement of the economic basis of the population".

J. Trémolières, in the third type of article, writes in volume 18, as he has done elsewhere before, to imply that food has a symbolic significance apart from its chemistry and that people are different from the nutritional statistics in which we sometimes purport to describe them. "Reference Man" must always be based on the particular community to which he is intended to be applied.

Finally, there are thirty-five pages in volume 17 by J. E. Gordon and N. S. Scrimshaw—surely written on an off day. This too is a philosophical exposition of their views on how to decide whether action planned to improve a

community's nutrition has done any good or not. "Essential components are to establish the goals of professional achievement for nutritional and allied variables, the means for their measurement, a standardisation of performance in their execution and quality control of the assembled data", write the authors or, alternatively, "the approach to evaluation of a nutrition action programme is ecologic, wherein epidemiologic method provides the means to measure effect".

If Dr Geoffrey Bourne, the indefatigable editor of the series, could persuade some of the contributors to knock off a word here and there, he might be able to get the publishers to reduce the price. **MAGNUS PYKE**

Ways with cells

New Techniques in Biophysics and Cell Biology. Edited by R. H. Pain and B. J. Smith. Vol. 1 pp. 249. (Wiley-Interscience: London and New York, September 1973.) £5.73.

THIS little book clearly emphasises how present-day biology depends on technical innovation. Like all multi-author works, it is heterogeneous in style and approach. Some of the eight chapters describe in detail the theory behind each technique but are thin on useful applications, whereas others list the various problems successfully tackled but with a minimum of theoretical background.

New Techniques begins with an account by J. Edwards of intercellular adhesion in which the Coulter counter is fully exploited to study aggregation of BHK 21 cells, which are the author's speciality. It is followed by a lucid and well-documented description by K. W. Jones of *in situ* DNA-RNA hybridisation, a technique which has already yielded valuable information on gene analysis during development. N. G. Maroudas concludes that there is no "best buy" among the different techniques for anchorage-based cell propagation. There is an authoritative chapter by R. G. Miller on cell separation by velocity sedimentation but the excellent theoretical introduction is not matched by sufficient examples of successful or possible applications of the technique. This is followed by the only two chapters on biophysics—one on circular dichroism as a probe for polysaccharide structure and the other on the application of mass spectrometry to

protein sequencing. P. L. Pearson's chapter on differential staining of mammalian chromosomes ends with a useful collection of recipes. The last chapter by S. P. Spragg on on-line computers is more useful for information obtained with physical methods than those of cell biology.

Looking at the book as a whole it is difficult to see the rationale for grouping these five methods of cell biology with two physical techniques, particularly as it is in its scope, will be useful to many biologists, but hope that the next volume will include techniques such as electron microscopic visualisation of genetic transcription or the application of NMR to biological problems.

J. R. TATA

Organic orbitals

The Organic Chemist's Book of Orbitals. By William L. Jorgensen and Lionel Salem. Pp. xii+305. (Academic (Harcourt Brace Jovanovich): New York and London, May 1973.) \$11.50.

THERE is a growing group of organic quantum chemists who believe that the symmetry properties and shapes of orbitals provide the most informative insight into the structure and reactivity of organic molecules. The concern of the authors of this book is to provide the requisite information in the form of electron density contour diagrams for 104 selected molecules of special interest, from hydrogen to norbornadiene. In each case there are three-dimensional computer-produced drawings for the occupied valence orbitals and for at least one unoccupied orbital, together with their group-theoretical symmetry and the orbital energy. These annotated drawings occupy nearly three-quarters of the book. There is a brief introductory account of molecular orbitals and their genesis from bond orbitals and group orbitals. The remaining pages consist of literature references and indications of the computational methods used (*ab initio* with extended basis sets, MINDO/2 or extended Hückel wave functions). The computer programs for the graphical output devices are not given, nor is there any indication of the effort in terms of programming and computer time.

Any chemist with a weakness for computer graphics should enjoy the pretty drawings produced. While not everyone will share completely the authors' enthusiasm for their usefulness, it is perhaps worth remembering that it can be argued that most progress in

theoretical organic chemistry has come through the contemplation of three-dimensional atomic arrangements in molecules. The extension of this approach to electrons is therefore within the tradition of mainstream organic chemistry.

V. GOLD

Subatomic interactions

The Pion-Nucleon System. By B. H. Bransden and R. G. Moorhouse. Pp. ix+538. (Princeton University: Princeton, July 1973.) \$20 boards; \$8.95 paper.

OF all elementary particle interactions, the πN system is undoubtedly the one which has been most extensively studied. This has resulted in a wealth of accurate data for pion-induced reactions, and natural accompaniments have been detailed phenomenological studies, model building, and the testing of theories. This is the field of Bransden and Moorhouse's graduate-level book, although it also contains much material (30–40%) about the techniques of hadron physics in general.

The contents, briefly, are as follows. The book opens with a discussion of basic quantities such as isospin, scattering amplitudes and cross-sections. These are illustrated in Chapter 2 by analyses of low-energy πN scattering and pion photoproduction. Chapter 3 is concerned with fixed- t dispersion relations, starting with a field theoretical derivation of forward dispersion relations for spinless particles, and then discussing applications to πN scattering. Analyticity is developed further in Chapter 4, which contains a review of the analytical properties of amplitudes for potential scattering, and then discusses the relativistic πN case, leading to the Mandelstam representation and partial wave dispersion relations. These four chapters are in general clearly written and straightforward, although the discussion of the Mandelstam representation concentrates exclusively on its analytical properties without a warning statement about the asymptotic behaviour necessary for its existence.

Baryon resonances are the subject of the next two chapters. The first, Chapter 5, contains a detailed account of resonance theory, and explains very clearly the general forms of resonant amplitudes and how they arise. The second half of this chapter describes how resonance parameters are deduced from data by phase shift analysis, and gives an assessment of the results that have been obtained. A somewhat surprising omission is the lack of any description of the actual data themselves, not even qualitatively. The second of these chapters, Chapter 6, discusses the classification of resonances in higher symmetries, starting

from the isospin group SU(2) and ending with the L-excitation quark model. There is a good physical discussion of group theoretical ideas, and extensive use is made of Young tableaux. Symmetry ideas are also the subject of Chapter 7 on current algebra and its sum rules. Chapter 8 contains a description of higher-energy two-body scattering, and in particular the ideas of Regge poles and duality, and in Chapter 9 the analyticity approach reappears in a review of some attempts at πN dynamical calculations. The final two chapters, 10 and 11, are concerned with πN inelastic scattering, and a detailed discussion of the form factors of the pion and nucleon, respectively. There are two appendices, on angular momentum and the formalism for pion photoproduction.

In reading this book I was conscious of a certain unevenness in the background required of the reader. For example, some knowledge of field theory is required to understand chapters 3 and 7, whereas isospin is (more than once) introduced without assuming any prior knowledge. This is also true of the style. Undoubtedly the best sections of the book are those where the discussion starts from a comparatively low level and the ideas are developed in detail with the necessary full explanation. Chapters 5, 6 and 11 are of this form, and all are excellent. Less successful are those sections where motivation is lacking, or where discussion terminates at an interesting point. An example of the latter is the discussion of resonance poles on page 185. An example of the former is the concept of "signature". I doubt whether someone meeting the idea, as introduced in section 8.4, for the first time would really appreciate its significance. Graduate students may also be alienated by the fact that words are frequently mentioned long before their meaning is explained; for example, "exotic" and "Veneziano model" (or, occasionally, not explained at all). The readers likely to obtain most from this book are thus those with some prior knowledge of strong interaction physics. They are not likely to be disappointed, for almost all aspects of πN physics are discussed. Certainly, all particle physics libraries should possess a copy.

The volume is well produced, and, since there is a paperback edition, not unreasonably priced. There is a considerable number of misprints, but fortunately most of these are trivial. Despite its typed format, it seems to have taken rather a long time to produce (the preface is dated April 1972) with a result that many of the references have been superseded, but that is probably inevitable in an advancing field like particle physics.

B. R. MARTIN

Embryos and ethics

The Mammalian Fetus in vitro. Edited by C. R. Austin. Pp. xii+388. (Chapman and Hall: London, July 1973.) £8.

THE *in vitro* culture of mouse and rat embryos during the early somite stages of development has little directly in common with the physiological problems of the premature human infant. It is the aim of *The Mammalian Fetus in vitro* to establish a connection by including both in a series of fields of study, each of which overlaps the next. To further this aim, as the editor explains in his introductory chapter, the meaning of the term 'foetus' has been extended backwards and forwards in time somewhat beyond its usual limits.

Culture of post-implantation stages of rodent embryos through the period of organogenesis is described by D. A. T. New, who himself developed most of the relevant techniques. Later organogenesis is particularly inaccessible in placental mammals, but can be studied in marsupials not *in vitro* but in the pouch. In his chapter on marsupial pouch young, G. B. Sharman presents a comprehensive compilation of data on a topic never previously reviewed. D. A. Nixon deals with the physiology of the mid-term foetus and placenta, mainly on the basis of experiments on foetal lambs. The same theme is considered, with particular reference to the development of an artificial placenta, by W. M. Zapol and T. Kolobow on the foetal lamb and by M. C. Macnaughton on the mid-term human foetus. The problems in this field are enormous, and it is abundantly clear that the vitri-
parity of Aldous Huxley's *Brave New World* is not yet in sight.

Moving on to the viable (or almost so) stage, C. H. M. Walker and B. J. N. Z. Danesh describe their artificial lung-kidney system as used on puppies and human infants, and E. Hey contributes an outstanding chapter on the problems of the premature baby, including respiration, thermoregulation, circulation and nutrition.

Some of the ethical problems involved are discussed in a final chapter, 'The Road Ahead', by R. A. McCance. Clearly the enormous strides that have been made in reducing perinatal mortality would have been impossible without extensive experimentation, on human as well as animal material. Sometimes the measures designed to save the lives of infants introduce new and unsuspected hazards: for example, the hundreds of babies suffering retinal damage and subsequent blindness as a result of excessive oxygen treatment in the early 1950s, or the many deaths from 'grey baby syndrome' a few years later, due to injudicious treatment with chloramphenicol.

Guidelines for the use of human foetuses for research have been laid down in the 1972 Report of the Advisory Group set up by the College of Obstetricians and Gynaecologists. Different ethical principles apply to viable and to non-viable infants; in the latter case, as also with animal experimentation, the problem is almost as much aesthetic as ethical. McCance regrets that the Advisory Group recommended that foetuses of more than 20 weeks' gestation should be considered viable, since he feels that this creates a 'shadowy period of 8 weeks' during which abortions can be performed, yet the use of the abortuses (almost certainly not viable) for experiments of any kind may become illegal. From the point of view of experiments which could involve pain and distress, the cut-off point should surely come earlier: a 19-week foetus, however non-viable, differs from a 6-day blastocyst in possessing all the mechanisms for feeling pain.

One wider ethical point is not con-

sidered in this volume. The survival rate of liveborn babies weighing between 1 and 1.5 kg at birth has been pushed up from 50% to 75%, and 20% even of those weighing less than 1 kg may now 'expect to survive the neonatal period with expert care'. Fortunately, the outlook for survivors has also improved: past reports on babies weighing less than 1.5 kg at birth suggested that more than half would be handicapped, but more recent reports indicate that 'with skilled nursing care less than 20% of the survivors will be seriously handicapped'. Even this figure makes a disturbingly high contribution to the total burden of physical and mental handicap. Should lung-kidney machines and all the other achievements of foetal and neonatal physiology be used to try to maintain viability in earlier and yet earlier premature infants? If not, where should the line be drawn?

As one might expect from Professor Austin's editorship, the volume is impeccably turned out.

ANNE McLAREN

View of the brain

The Understanding of the Brain. By John C. Eccles. Pp. xv+238. (McGraw-Hill: New York and London, May 1973.) £1.95.

THIS short book is based upon a series of lectures delivered at Indiana University early last year. Its aim is to provide an outline of recent neurophysiological work of special relevance to our understanding of brain function and, incidentally, to convey the author's somewhat idiosyncratic views on the relations of brain, mind and consciousness.

Professor Eccles makes it clear that his book is to be regarded not as a text or reference work but as a personal communication limited to aspects of the subject which he regards as of particular interest or importance. These aspects are covered in six longish chapters, dealing respectively with neurones and the nerve impulse, peripheral synaptic transmission, synaptic transmission and pathways in the brain, the control of movement, neurogenesis and the neural basis of learning, and finally, brain, speech and consciousness. A list of selected references, mostly recent though certainly not exhaustive, is appended to each chapter.

The material covered in the earlier chapters is presented with the skill and cogency we have come to expect from Professor Eccles; his account of peripheral transmission, in particular, would seem outstandingly well balanced, particularly when his own changes of view over the years are borne in mind. So, too, is the chapter on the central mechanisms of transmission, in which

he goes beyond the experimental data in a praiseworthy attempt to formulate general principles governing central nervous activity. Although it is beyond my competence to assess these chapters at an expert level, they seem to me pertinent, well presented and fair.

The chapter on neurogenesis brings together, in a brief compass, much recent experimental material considered in relation to the pioneer work of such men as Weiss and Sperry. The author defends strongly the view that the developing brain can no longer be viewed as a randomly organised structure moulded by use to an appropriate design but rather as a system in which the basic patterns of connection are established prior to their being called into action. This conclusion obviously has important implications for many problems in genetic neurology.

In his account of movement and its control, Professor Eccles has a great deal more to say about the cerebellum than the motor cortex, though he has little use for those who have attempted to understand its functions on the basis of computer models. For this reason, he makes no reference to the work of Marr and others, which many have found extremely stimulating. It is a pity, perhaps, that this whole approach is dismissed so cavalierly.

The final chapter on mind and brain is inevitably controversial. Rightly, the author devotes considerable attention to the work of Sperry on 'split-brain' patients. As is well known, a variety of fascinating disconnections between the activities of the two cerebral hemispheres can be demonstrated in these patients, such that each may under

appropriate circumstances operate at a high-order level independently of its fellow. Although the right hemisphere seems to be largely, if not wholly, devoid of linguistic expression, it is nonetheless almost certainly unjustified to treat it, as does Professor Eccles, simply as a "computer" lacking any direct liaison with consciousness. No one who has watched a split-brain patient performing complex visual discrimination and recognition tasks processed wholly by the right hemisphere could possibly doubt the presence of consciousness in the sense in which the term is ordinarily used. It is difficult not to suspect that Professor Eccles's view in the matter is governed, in part at least, by extrascientific considerations.

This book has a glossary though no index. It should make stimulating reading for medical students and others seeking a concise and up-to-date account of several important aspects of contemporary neurophysiology. At the same time, its intentionally selective character will obviously limit its usefulness as a formal text. O. L. ZANGWILL

Social wasps and loners

Wasps: An Account of the Biology and Natural History of Solitary and Social Wasps, with Particular Reference to Those of the British Isles. By J. P. Spradbery. Pp. xvi+408 (28 plates). (Sidgwick and Jackson: London, September 1973; University of Washington: Seattle, November 1973.) £8.50; \$17.50.

WHAT is a wasp? Dr Spradbery adopts a very narrow definition to include only the members of the Vespoidea, a group which consists of the familiar social wasps and a number of solitary species. In a recent work of very similar title (*The Wasps*, University of Michigan Press, 1970), Evans and Eberhardt take a much wider definition to include all aculeate, or stinging, Hymenoptera, with the exception of the ants and bees. Such a grouping includes the majority of solitary forms (particularly of the Sphecoidea), which have been studied by so many insect ethologists since the early work of J. H. Fabre. Of course, it is quite reasonable to take the more restrictive view, but even Dr Spradbery in his text uses the term frequently in a more general sense.

Taking the Vespoidea then as his group, Dr Spradbery gives us an excellent monographic treatment based mostly on the British species, but with widespread reference to other faunas and to literature otherwise difficult for the non-specialist to obtain. The first part of the book is devoted to a good general discussion of the group, with emphasis on structure and function. There follows a chapter on the solitary species, of the family Eumenidae, concerned mainly with comparative

behaviour studies. It is perhaps here that some comparison with the more diverse sphecoid wasps might have been included with advantage. The next eight chapters form the core of the book and are devoted to a thorough review of the behaviour, particularly in terms of nesting, foraging and social organisation, and the population ecology of the British Vespidae, the social wasps.

As would be expected from the author's previous publications, there is a first-class, balanced discussion of many problems, including the controversial field of caste determination. A chapter is devoted to the parasites, predators and commensals associated with wasp colonies. This necessarily is drawn from a wide variety of published records, at least some of which need judging with caution. Chapter twelve is entitled "The Impact of Wasps on Man" and is a useful summary, especially of the sting and its pharmacology. It may come as some surprise to discover that during the period 1949 to 1969, thirty cases were recorded of people in England and Wales who died as a direct result of being stung by wasps! The main part of the book concludes with two chapters of a less detailed nature, which discuss the 800 or so social vespid species of the world and the possible paths and mechanisms of the evolution of such complex societies.

The book concludes with four very useful appendices. The first is concerned with the taxonomy of the British species and gives good illustrated keys for the identification of the twenty-nine known species. A further appendix gives helpful information on methods of studying, collecting and preserving wasps. The final appendix gives the first published distribution maps for all British species, compiled by the Biological Records Centre, Monks Wood. Clearly these are very incomplete, but their very inadequacy may foster an interest in the group, which is essential for the accumulation of further information.

The standard of line illustrations throughout the book is generally very high. The half-tone plates are in some cases less satisfactory. Most show live wasps or their nests, and others show structural details. A number of good colour plates are included, but those illustrating mounted specimens are rather disappointing. The individual insects are mostly too small to show useful detail and some are very poorly reproduced. Most unfortunately the colour and black-and-white plates are inconsistently laid out, with blank pages interspersed irregularly, for no apparent reason. The legends are often very difficult to associate with the correct figures, and sometimes even hard

to find.

In conclusion this is clearly a very valuable and exhaustive work which should be read not only by the small band of research workers interested in wasps, but also by a wider group of general ethologists and ecologists. It will without doubt for long be a standard work in its field.

M. F. CLARIDGE

Straight dislocations

Introduction to Anisotropic Elasticity Theory of Dislocations. By J. W. Steeds. Pp. ix+274. (Clarendon: Oxford; Oxford University: London, May 1973.) £8.35.

IN this book Steeds presents a concise, very clearly written account of the elastic theory of straight dislocations in anisotropic media. He concentrates exclusively on those cases whose solution can be analytically expressed, and he shows that these comprise such a large number of cases that the algebraic effort is worthwhile. In short, Steeds 'professionalises' the subject so that a research student with the aid of this book should be able to make intelligent use of anisotropic elasticity, whereas before the book was available most research students (and their supervisors) would founder.

The book contains explicit solutions for a large number of commonly occurring dislocations, and a table at the end lists them so that one can see at a glance whether or not a particular problem is solved. Furthermore, a very clearly written chapter outlines the relationship between the two-dimensional (that is, straight dislocation) solutions and the three-dimensional solutions; it also covers dislocations in uniform motion.

The book is intended to be a reference work, and it has extensive tables of parameters for different crystals. As a reference book, it gains greatly from its clarity and strict deductive approach. As a textbook, it suffers somewhat from these features: students will not easily gain from it a feel for when anisotropic elasticity is important and when it is not; and one would perhaps like guidance on the use of computers to solve some of these problems. In my experience, it is possible to write a computer program which works reliably and which obviates the necessity for algebra or thought—although of course it does not give the kind of insight obtainable from analytic formulae. The computer program itself, however, requires an investment of time which will not pay off unless extensive calculations are envisaged—the one-off calculation is better done by algebra. So there is no doubt that any laboratory concerned with the properties of dislocations should have this book on its library shelves.

L. M. BROWN